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CHROMOSOMAL CONFIGURATIONS OF OENOTHERA SPECIES AND CROSSES AND THEIR PROBABLE SIGNIFICANCE

J. THERON ILLICK

The purpose of this paper is to tabulate all chromosomal configurations of the *Oenothera* species and crosses that have so far been observed and to discuss the significance of chromosomal cohesion.

The tabulations of each form include the name of the investigator, the year reported, and, if available, the source of material and the genetical constitution of each. The writer does not claim that these tables are complete in every instance, but an attempt is made to cover all the work available to date.

Such a series of tables, it is felt, would be of great advantage in any attempt to understand the significance of closed circles so characteristic of the *Oenothera* species. In connection with each table there is given a brief discussion of the forms therein listed. In these discussions there are emphasized in particular two points based upon the data presented in these tables: (1) the variability in chromosomal configurations in any particular *Oenothera* species; and (2) the assumption that closed circles are due to genic action and therefore inherited as are phenotypical characters.

Table I (7 pairs) consists of nineteen different species, each of which, as observed by different investigators, has shown a complete pairing of the fourteen chromosomes. These nineteen Oenotheras consist of six species, three mutants, one half-mutant, six segregates, and three hybrid derivatives, some of which have been recognized

TABLE I

CHROMOSOME CONFIGURATION WITH 7 PAIRS

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
lamarckiana*	1924, BOEDIJN	Original strain of DEVRIES	Unstable; isogamous (pollen and egg have same qualities); gives about $1\frac{1}{2}\%$ "mutants"; of its gene mutations, none has repeated itself except *funifolia* which appeared in *O. pratincola* (1919) and in *lamarckiana* (**56**); complex *velans.gaudens*. Also shows ⊙ 12 and 1 pair as most common configuration; or ⊙ 10 and 2 pairs; or 3 pairs and chain; or chain of 14. (There has been expressed some doubt as to correctness of the presence of 7 pairs.)
pervirens*	1929, ILLICK	SHULL	Mutant by SHULL; assumed to be the same genetically as *lamarckiana* except it has no red pigment and shows a higher percentage of germination (**56**). Also shows ⊙ 12 and 1 pair as most common condition; or ⊙ 14.
blandina	1925, CLELAND	DEVRIES	Direct mutant of *lamarckiana;* first appeared from cross between *lata* and *semilata* and is homozygous in crosses; no lethals and is constant except for occasional mutant *spiralis* (**30**); homozygous race (DE VRIES); STOMPS (**62**) says it possesses a greater faculty for mutation than had hitherto been believed; belongs to *velutina* class.
rubrinervis*	1908, GATES	GATES	HÅKANSSON calls it half-mutant of *lamarckiana;* in 1907 gave the mutant *rubricalyx;* gives homozygous segregate *deserens* and in appearance is difficult to distinguish from *deserens;* CLELAND calls his forms homozygous strains; GATES claims it is heterozygous for *laeta.velutina* (**25**). Also shows ⊙ 6 and 4 pairs as the more common configuration.
deserens	1925, CLELAND 1930, HÅKANSSON	DEVRIES ?	Alethal segregate from half-mutant *rubrinervis* and difficult to distinguish from *rubrinervis;* homozygous race (DEVRIES); belongs to *laeta* class.
fragilis	1929, HOEPPENER and RENNER	?	A *deserens*-like homozygous form from all hybrids containing *subvelans*.

* Indicates variable configuration of chromosomes.

TABLE I—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
rubricalyx sulfurea	1929, ILLICK	SHULL	Hybrid derivative of *rubricalyx*-budded *brevistylis* (or *rubricalyx*-budded *nanella*)×*franciscana sulfurea*.
latifrons	1928, CLELAND	SHULL (DAVIS)	Alethal segregate from half-mutant *rubricalyx;* homozygous race (DE VRIES).
hookeri	1924, SCHWEMMLE 1928, CLELAND 1929, HOEPPENER and RENNER	? DEVRIES ?	Very stable; has given *franciscana* (**26**) which is also a stable form; wild species; isogamous; complex $^{h}hookeri.^{h}hookeri$.
franciscana*	1928, CLELAND 1929, KULKARNI 1929, HOEPPENER and RENNER 1931, LELIVELD	SHULL (DAVIS) DAVIS (BARTLETT) ? STOMPS	Arose as segregate from *hookeri* (**26**); very stable, genetically more so than *grandiflora;* has never thrown any mutants and has shown high percentage of pollen and seed fertility; CLELAND (**5**) stresses it as pure species; GATES (**27**) claims it must be heterozygous for a number of characters. Also shows ⊙ 4 and 5 pairs; or chain of 4 or more.
franciscana* sulfurea	1928, CLELAND 1929, ILLICK	SHULL (DAVIS) SHULL (DAVIS)	Hybrid derivative of *franciscana*×*biennis;* similar to *franciscana* genetically except it has sulfur flowers; non-disjunction high (**7**). Also shows ⊙ 12 and 1 pair as less common configuration.
franciscana sulfurea (dwarf seg.)	1928, EMERSON	DAVIS	*Franciscana sulfurea,* selfed, gives many parental forms and about 1/3 dwarf segregates as this; this segregate "is apparently homozygous for all characters."
grandiflora* Ait.	1929, GERHARD	?	"Complex heterozygous," whose complexes, *truncans.acuens,* are functional both in pollen and egg cells; *acuens* is viable in homozygous condition, while *truncans* is not; consequently *grandiflora* does not breed true but splits off in each generation 1/3 *ochracea* (*acuens.acuens*). *Grandiflora* forms twin hybrids with other species. Also shows ⊙ 14; or ⊙ 12 and 1 pair.
ochracea	1929, GERHARD	?	See above; complex *acuens.acuens*.

* Indicates variable configuration of chromosomes.

TABLE I—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
grandiflora* B	1909, DAVIS 1928, CLELAND 1929, ILLICK	DAVIS SHULL (DAVIS) SHULL (DAVIS)	Very stable wild species; has complex *acuens.truncans*. Also shows ⊙ 4 and 5 pairs; or ⊙ 14.
lutescens	1929, HOEPPENER and RENNER	HERIBERT-NILSSON	Hybrid derivative of *suaveolens*×*biennis* from culture of HERIBERT-NILSSON of *lata*-like plants in 1912; larger flowers than *lamarckiana;* complex *flavens.flavens*.
flava*	1926, OEHLKERS	OEHLKERS	F_1 segregate of *O. suaveolens*×*strigosa;* segregates many new types. Also shows ⊙ 4 and 5 pairs.
twin hybrid of recidiva	1929, KULKARNI	DAVIS	Twin hybrid from *recidiva*, selfed; stunted gray non-flowering plant.
purpurata	1929, RUDLOFF	?	Species.

*Indicates variable configuration of chromosomes.

genetically to be either stable or unstable, homozygous or heterozygous.

Eight of the total of nineteen have shown variable chromosomal configurations. Three of these are *rubrinervis*, *franciscana*, and *grandiflora* Ait. GATES (**25**) calls *rubrinervis* heterozygous for *laeta.velutina* and has observed two types of configurations, 7 complete pairs and 4 pairs and ⊙[1]6, while CLELAND (**8**) calls his strains homozygous and observes 4 pairs and ⊙ 6. *Franciscana*, a segregate from *hookeri* and very stable genetically, CLELAND (**5**) stresses as a pure or homozygous species, while GATES claims his plants must be heterozygous for a number of factors. The chromosomal configurations for this species, seen by four different observers, consist either of 7 pairs, 5 pairs and ⊙ 4, or a chain of 4 or more. *Grandiflora* Ait., GERHARD (**35**) calls a "complex heterozygote" and has seen three different configurations, 7 pairs, 1 pair and ⊙ 12, and ⊙ 14. The complex present is *truncans.acuens* and is the same as for *grandiflora* B, which shows either 7 pairs, 5 pairs and ⊙ 4, or ⊙ 14.

Assuming that these statements are correct, it seems safe to con-

[1] Throughout this paper, this symbol indicates a closed circle of chromosomes.

clude that neither heterozygosity nor homozygosity is concerned in the formation of closed circles.

Fifteen of the total of nineteen species may be divided into three groups from the standpoint of their known genetical relationship. In group 1, we have *lamarckiana*, which has been reported once to have shown 7 complete pairs, the mutants of *lamarckiana*, *pervirens*, and *blandina*, and half-mutant *rubrinervis;* also *deserens* (an alethal segregate from *rubrinervis*), *fragilis* (a *deserens*-like homozygous form), and *rubricalyx sulfurea* and *latifrons*, both indirectly related to *rubrinervis* through *rubricalyx*. In group 2 there are *hookeri* with 7 complete pairs, *franciscana*, a segregate from *hookeri*, *franciscana sulfurea*, a hybrid derivative of *franciscana*×*biennis*, and *franciscana sulfurea*, a dwarf segregate of *franciscana sulfurea*. In group 3, *grandiflora* Ait. has shown 7 pairs and gives the segregate *ochracea*. *Grandiflora* B is the same as *grandiflora* Ait., at least so far as its reported complex is concerned. The ancestral forms of the other four Oenotheras, so far as is known, have not shown complete pairing of their chromosomes.

From the data presented, it appears that any one chromosomal configuration is not constant for any one species but only relatively constant. In no instance, moreover, so far as known, were there observed any phenotypical characters corresponding to these various chromosomal configurations.

It is also to be noted that the members of each group into which fifteen of the nineteen individuals can be arranged, not only have shown 7 pairs of chromosomes but are known to be genetically related. Such a characteristic might well be interpreted as being due to an inherited factor as are the phenotypical characters.

Table II (5 pairs and ⊙ 4) includes a total of six different Oenotheras consisting of one species, one mutant, one half-mutant, and three segregates. They all have also shown other configurations. Three of the six have not been mentioned in table I.

Grandiflora is considered a very stable species; *franciscana* is said by GATES (**26**) to be heterozygous, while by CLELAND (**5**) it is called a pure species; *rubricalyx*, pure, is spoken of as a perpetual heterozygote possessing one of the zygote lethals of *lamarckiana;* and *flava* is unstable, segregating many new types. It would appear, therefore,

that genetical stability does not prevent the formation of closed circles, nor is it likely that heterozygosity is a factor in their forma-

TABLE II

CHROMOSOME CONFIGURATION WITH 5 PAIRS AND ⊙ 4

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
grandiflora* B	1919, CLELAND	SHULL	See table I. Also shows 7 pairs; or ⊙ 14.
franciscana*	1922, CLELAND	DAVIS (BARTLETT)	See table I. Also shows 7 pairs; or chain of 4 or more.
	1928, KULKARNI	DAVIS (BARTLETT)	
	1929, HOEPPENER and RENNER	?	
	1931, LELIVELD	STOMPS	
aurata*	1928, CLELAND	SHULL	Segregate from *franciscana*×*biennis*, in other words, an F_2 from cross between *velaurea* and sulfur (SHULL); breeds true; has *lamarckiana* in its ancestry. Also shows ⊙ 12 and 1 pair; or ⊙s 10 and 4.
	1929, ILLICK	SHULL	
rubricalyx,* pure	1927, SHEFFIELD	GATES	Mutant (Mendelian dominant) which occurred in culture of *rubrinervis* in 1907; has one of the zygote lethals found in *lamarckiana* and, like mut. *rubrinervis* and mut. *erythrina*, is a perpetual heterozygote which segregates in a 2:1 ratio in every generation into heterozygous *rubricalyx* and homozygous *latifrons* (from germ cells free of zygote lethals). Complex *rubens.tingens*. Also shows 4 pairs and ⊙ 6; or ⊙s 6 and 8; or variable.
	1929, GATES and SHEFFIELD	GATES	
flava*	1929, OEHLKERS	OEHLKERS	See table I. Also shows 7 pairs.
rubrisepala*	1930, HÅKANSSON	?	Half-mutant from *lamarckiana* (plants with unbalanced lethal condition). Also shows 4 pairs and ⊙ 6.

* Indicates variable configuration of chromosomes.

tion. The variations in configurations, moreover, are not known to be related to any observed phenotypical characters.

On the other hand, it is to be noted that those forms that are

known to be genetically related, as *franciscana* and *hookeri* (7 pairs), *franciscana* and *aurata* (5 pairs and ⊙ 4), *aurata* and *lamarckiana* (1 pair and ⊙ 12), and *rubricalyx* and *rubrinervis* (4 pairs and ⊙ 6), have each one known chromosomal configuration in common, which

TABLE III

CHROMOSOME CONFIGURATION WITH 4 PAIRS AND ⊙ 6

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
rubrinervis*	1908, GATES 1925, CLELAND 1928, CLELAND 1928, HÅKANSSON 1929, ILLICK	GATES SHULL SHULL (DAVIS) ? SHULL	See table I. HÅKANSSON studied ovules. Complex *paenvelans.subvelans*. Also shows 7 pairs as less common configuration.
rubricalyx,* pure	1927, SHEFFIELD 1929, GATES and SHEFFIELD	GATES GATES	See table II. GATES and SHEFFIELD studied megaspore development. Also shows 5 pairs and ⊙ 4; or ⊙s 6 and 8; or variable.
rubrisepala*	1928, HÅKANSSON	?	See table II. Studied ovules. Also shows 5 pairs and ⊙ 4.
erythrina	1928, CLELAND	DAVIS	Half-mutant of *lamarckiana* and produces *decipiens* which is a homozygous race with no lethals and which breeds true and throws no mutants; perpetual heterozygote.
stenophylla* (selfed)	1932, ILLICK	SHULL	Mutant by DEVRIES from *lamarckiana*; *stenophylla* × *angustifolia* gives *lamarckiana*; narrow leaf. Also shows ⊙ 12 and 1 pair as less common configuration.
"mut. sulfurea"	1928, CLELAND	SHULL	All showed this configuration except one 21-chromosome plant.

* Indicates variable configuration of chromosomes.

permits the assumption that closed circles are the result of genic action.

Table III (4 pairs and ⊙ 6) includes six Oenotheras consisting of three mutants and three half-mutants, four of which show variable chromosomal configurations. One of these four, *stenophylla*, has not been mentioned in previous tables. *Erythrina*, a perpetual heterozygote and half-mutant of *lamarckiana*, and "mut. *sulfurea*" are the only ones which have thus far shown only single types of chro-

mosomal configurations. With the appearance of these variable chromosomal configurations no observed phenotypical changes are known to have been recorded for the individuals concerned.

TABLE IV

CHROMOSOME CONFIGURATION WITH 3 PAIRS AND ⊙ 8

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
lamarckiana*	1931, LELIVELD	STOMPS	See table I. Configuration here is 3 pairs and chain; also shows ⊙ 12 and 1 pair as most common configuration; or ⊙ 10 and 2 pairs; or chain of 14; or 7 pairs.
rubricalyx Afterglow	1925, CLELAND 1931, EMERSON	SHULL ?	CLELAND (13) believes this "Afterglow" of SHULL's arose through segmental interchange in pure *rubricalyx*. Complex modified *velans.blatifrons*.
germanica*	1931, LELIVELD	STOMPS	Offspring of BOEDIJN's plants. Configuration here consists of 3 pairs and loose chromosomes; also shows ⊙ 14; or ⊙ 12 and 1 pair; or ⊙ 10 and 2 pairs, these three configurations "in one and the same slide" (46).
distans	1930, HÅKANSSON	?	"From 2n+1 *nitens*" (18); also called 2n+1 or 15 chromosome mutant (BOEDIJN) and (GATES).
simplex elongata	1930, HÅKANSSON	?	"A segregate of *simplex* from 2n+1 *oblonga*" (18); "*simplex* agrees with mut.*velutina* (*blandina*) in having very few empty seeds and in not giving twin hybrids. While *blandina* forms hybrids of *velutina* type, the hybrids of *simplex* are of *laeta* type; also *simplex* retains the mutability of *lamarckiana*, which *blandina* has lost" (30).

* Indicates variable configuration of chromosomes.

It should be noted that *rubrinervis*, with 4 pairs and ⊙ 6, gives mut. *rubricalyx* also with 4 pairs and ⊙ 6; and *stenophylla* with 1 pair and ⊙ 12 is a mutant from *lamarckiana* which also commonly shows a similar configuration. These instances indicate that inherited factors are possibly involved in the production of closed circles as are phenotypical characters.

Table IV (3 pairs and ⊙ 8) includes five Oenotheras consisting of two species and three segregates. Two of the five, it will be noted, have shown variable configurations. One of these two, *germanica*, has not been mentioned in the previous tables. It is included in this table although the complete configuration was not observed. It showed 3 pairs and loose chromosomes. Further study might make it necessary to place it with another group of configurations.

Germanica is interesting in that LELIVELD (**46**) found three different configurations (⊙ 14, ⊙ 12 and 1 pair, and ⊙ 10 and 2 pairs) "in one and the same slide." It would therefore be difficult to explain closed circles in *germanica* on the basis of observed genetical behavior. These three configurations of *germanica* in the same slide, LELIVELD claims are a "consequence of fixation." Concerning this point also see page 43 of this paper.

Rubricalyx Afterglow is reported to have come from *rubricalyx* by segmental interchange (CLELAND **13**), and *rubricalyx* is a mutant of *rubrinervis* which in turn is a half-mutant of *lamarckiana*, and since *rubricalyx* Afterglow and *lamarckiana* have shown one configuration common to both (3 pairs and ⊙ 8), it might be supposed that similar heritable factors are involved in each instance.

The two forms which have shown more than one type of configuration have never been known to show any phenotypical variations corresponding to these configurations.

Table V (2 pairs and ⊙ 10) includes four individuals consisting of three species and one hybrid derivative, all of which have shown variable chromosomal configurations. Two of these were not included in the previous tables.

Lamarckiana, which is unstable genetically and heterozygous, has been said to have shown to date five different chromosomal configurations; *muricata*, which is stable genetically and permanently heterozygous, has shown three different chromosomal configurations; while *germanica* has shown three types of configurations, ⊙ 14, ⊙12 and 1 pair, and ⊙ 10 and 2 pairs, "in one and the same slide" (LELIVELD **46**), as well as 3 pairs and loose chromosomes.

In no instance where variation in configurations occurred was there observed any phenotypical changes with which they might be associated.

Table VI (1 pair and ⊙ 12) includes twenty-nine different Oenotheras consisting of eleven species, eleven mutants, five hybrid derivatives, and two segregates. Sixteen of these show variable chromosomal configurations, seven of which have not been previously mentioned.

TABLE V

CHROMOSOME CONFIGURATION WITH 2 PAIRS AND ⊙ 10

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
lamarckiana*	1931, LELIVELD	STOMPS	See table I. Five cases of this configuration seen. Ovules and pollen studied by LELIVELD. Also shows ⊙ 12 and 1 pair; or chain of 14; or 3 pairs and chain; or 7 pairs.
"heterozygous for 7 characters"*	1928, CLELAND	SHULL	One plant showed this configuration and another showed ⊙12 and 1 pair.
germanica*	1931, LELIVELD	STOMPS	See table IV. Besides this configuration it also shows ⊙ 14; or ⊙ 12 and 1 pair, all three seen in same slide. It also shows 3 pairs and loose chromosomes.
muricata*	1931, LELIVELD	STOMPS	Stable genetically; heterogamous (pollen and egg carry different qualities); possibly from *biennis* or simpler form; has segregated out *novae-scotiae;* called permanent heterozygous wild species (**10**); small flowers and microspores. Complex *curvans.rigens.* Also shows 1 pair and chain of 12; or ⊙ 14.

* Indicates variable configuration of chromosomes.

The presence of such variations again emphasizes the fact that chromosomal configurations are not necessarily fixed for any one species. With the appearance of such variations in configurations, moreover, there have been recorded no corresponding phenotypical differences which would permit the conclusion that closed circles are related to any particular phenotypical character.

Eighteen of the twenty-nine of this table can be placed genetically into four groups depending upon common parentage. In each instance the individual members show one or more different configura-

TABLE VI

CHROMOSOME CONFIGURATION WITH 1 PAIR AND ⊙ 12

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
lamarckiana*	1925, CLELAND	SHULL (DE VRIES)	See table I. *Lamarckiana* of HERIBERT-NILSSON is white-nerved and by RENNER called r-*lamarckiana*. HÅKANSSON in 1928 studied ovules. LELIVELD studied both pollen mother cells and ovules. Also shows ⊙ 10 and 2 pairs; or chain of 14; or 3 pairs and chain; or 7 pairs.
	1926, HÅKANSSON	HERIBERT-NILSSON	
	1928, HÅKANSSON	?	
	1929, CLELAND	RENNER (r-*lamarckiana*)	
	1929, CLELAND	DEVRIES	
	1929, ILLICK	SHULL (DE VRIES)	
	1931, LELIVELD	STOMPS	
mut. from lamarckiana	1932, ILLICK	SHULL	Gene mutant by SHULL.
vetaurea supplena (selfed)	1932, "	"	"Old gold" and "double flower" from *lamarckiana*.
angustifolia	1932, "	"	Gene mutant from *lamarckiana* by DEVRIES; *angustifolia*×*stenophylla* gives *lamarckiana;* narrow leaf.
pervirens*	1929, "	"	See table I. The one of 1932 was a selfed individual and showed ⊙ 14 and ⊙ 12 and 1 pair in cells four sections apart. Also shows 7 pairs.
	1932, "	"	
stenophylla* (selfed)	1932, "	"	See table III. Also shows ⊙ 6 and 4 pairs as more common condition.
bullata, pure	1932, "	"	Gene mutant from *lamarckiana* by SHULL.
acutifolia	1931, BRITTINGHAM	"	Gene mutant from *O. lamarckiana* cross-bred line maintained for 23 years; mutant gene is from outside first linkage group; characterized by narrower, more sharply pointed rosette leaf and reduction in amount of crinkling; closely resembles parent in floral characters and breeding phenomena. Fifth gene mutation to give typical monohybrid ratios.
lamarckiana cruciata	1929, CLELAND and OEHLKERS	OEHLKERS	Variety of *lamarckiana;* has complex *velans.gaudens*.
lamarckiana sulfurea	1932, ILLICK	SHULL	Hybrid derivative from *lamarckiana* ×*biennis*.

* Indicates variable configuration of chromosomes.

TABLE VI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
fallax	1928, HÅKANSSON	?	Hybrid from *lamarckiana*×*biennis*, Chicago (**37**). Also see table XII for this cross.
suaveolens* (yellow)	1926, OEHLKERS 1928, CLELAND 1929, ILLICK	OEHLKERS RENNER (DE VRIES) SHULL	Resembles *biennis* in breeding behavior in throwing unlike reciprocal hybrids; wild type. Complex *flavens.albicans*. Also shows ⊙ 14.
suaveolens (sulfur)	1929, " 1929, CLELAND and OEHLKERS	" OEHLKERS	Mutant from wild yellow-flowered form. Complex *flavens.albicans*.
albata*	1926, OEHLKERS	OEHLKERS	F_1 segregate of *O. suaveolens*×*strigosa;* breeds true except in its descendents. Also shows ⊙ 14.
pratincola,* Bartlett	1929, ILLICK	SHULL	Wild species; closely resembles *biennis* genetically in giving unlike reciprocal hybrids (egg and sperm lethals). Also shows ⊙ 14.
simulans*	1929, KULKARNI	DAVIS	Mutant strain of *pratincola* (Bartlett). Only two types of gametes produced. Also shows ⊙ 14.
franciscana sulfurea*	1923, 1924, CLELAND	"	See table I. Also shows 7 pairs as more common condition.
aurata*	1928, CLELAND	SHULL	See table II. Also shows ⊙ 4 and 5 pairs; or ⊙s 10 and 4.
gigantea (diploid)	1928, HÅKANSSON	?	Mutant from Swedish *lamarckiana* by HERIBERT-NILSSON, 1907; nondisjunction frequent (**30**); HÅKANSSON studied ovules.
"heterozygous for 7 characters"*	1928, CLELAND	SHULL	One plant showed this configuration and another showed ⊙ 10 and 2 pairs.
Oe. (rc. V. 0)	1932, ILLICK	"	Will be expected to segregate for "outside in."
strigosa*	1926, OEHLKERS	OEHLKERS	"Most nearly related to *hookeri*, from which it differs in its small flowers, and in its foliage" (**26**). When used as male parent and crossed with *lamarckiana*, it gives twin hybrids, *laeta* and *velutina;* wild species and permanently heterozygous. Complex *deprimens. stringens*. Also shows ⊙ 14 as more common condition.

* Indicates variable configuration of chromosomes.

TABLE VI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
cockerelli*	1926, OEHLKERS	OEHLKERS	Wild species; permanently heterozygous; when used as male parent and crossed with *lamarckiana* it gives twin types, *laeta.velutina;* small flowers and microspores. Complex *elongans.curtans*. Also shows ⊙ 14 as more common condition.
ammophila*	1927, SHEFFIELD 1932, ILLICK	GATES (RENNER) SHULL	Strain of unknown origin; very similar to *eriensis*. "Described by FOCH 1904 from German sand-dunes where it had been introduced from America" (31). Complex of SHEFFIELD's plants *rigens. percurvans*. Also shows ⊙ 14 as less common condition in several collections from one plant.
rubristachys	1928, HÅKANSSON	?	Studied the ovules.
germanica*	1931, LELIVELD	STOMPS	See table IV. Also shows ⊙ 14; or ⊙ 10 and 2 pairs; or 3 pairs and loose chromosomes.
grandiflora* Ait.	1929, GERHARD	DEVRIES	See table I. Also shows ⊙ 14; or 7 pairs.
muricata*	1931, LELIVELD	STOMPS	See table V. LELIVELD saw six cases of chain of 12 and 1 pair. Also shows ⊙ 14; or chain of 10 and 2 pairs.
chicagoensis	1931, CLELAND	From three different countries	Name given by RENNER to the "*biennis* Chicago" of DEVRIES.

* Indicates variable configuration of chromosomes.

tions which have been observed in the parents concerned. Sufficient data on this point are not available for the other eleven of these twenty-nine forms.

The four groups are as follows: (1) The gene mutants from *lamarckiana* (as mut. from *lamarckiana*, *vetaurea supplena*, *angustifolia*, *pervirens*, *stenophylla*, *bullata*, and *acutifolia*), a variety of *lamarckiana* (*lamarckiana cruciata*), and the hybrid derivatives of *lamarckiana*×*biennis* (as *lamarckiana sulfurea* and *fallax*), all have shown one or more configurations like *lamarckiana*. Two of these

have been mentioned previously in tables I and III respectively. (2) *Suaveolens* (sulfur) is a mutant from *suaveolens* (yellow), both of which have one configuration in common (1 pair and ⊙ 12). *Albata*, an F_1 segregate of *O. suaveolens*×*strigosa*, shows two types of configurations both of which resemble the types shown by its one parent, *suaveolens* (⊙ 14 and ⊙ 12 and 1 pair). (3) *Simulans*, a mutant from *pratincola* (Bartlett), also shows two types of configurations both of which resemble those of the parent, *pratincola* (⊙ 14 and ⊙ 12 and 1 pair). (4) *Franciscana sulfurea*, a hybrid derivative of *lamarckiana* ×*biennis*, shows one configuration, 7 pairs, like one of its parents, *franciscana;* and *aurata* resembles one of its parents, also *franciscana*, in showing 5 pairs and ⊙ 4.

In a letter from Professor G. H. SHULL under date of June 6, 1929, he says, "in the breeding behavior of this plant (27216 (17) *lamarckiana sulfurea*) the results are the same as if it were an *erythrina* instead of a *lamarckiana*." *Erythrina* has shown a configuration of ⊙ 6 and 4 pairs and *lamarckiana sulfurea* a ⊙ 12 and 1 pair. In this instance the breeding behavior of the individual has no apparent relation to the chromosomal configuration. In other words, the fact that any two plants exhibit the same breeding behavior does not necessarily permit the conclusion that their chromosomal configurations are identical. Resemblances in chromosomal configurations between offspring and parents as previously shown, on the other hand, permit the assumption that closed circles are inherited as are ordinary phenotypical characters.

Table VII (⊙s 6 and 8) includes five individuals consisting of one species, three mutants, and one segregate. None of these has been previously mentioned. Four of the five show variable chromosomal configurations.

If *muricata* arose from *biennis*, as has been suggested, then four of the individuals in this table are genetically of the same strain, that is, *biennis*. *Biennis sulfurea* and *biennis cruciata* are both mutants of *biennis* and show at least one of the chromosomal configurations of the parent. The former shows both configurations of *biennis*, as does *novae-scotiae* which is a segregate of *muricata*. The origin of *rubricalyx* from *rubrinervis*, and their possession of a common configuration, have been mentioned in table II.

Such similarity in chromosomal configuration between parent and offspring should not be considered a mere coincidence. On the other hand, it indicates a possible hereditary mechanism for the formation

TABLE VII

CHROMOSOME CONFIGURATION WITH ⊙s 6 AND 8

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
biennis L.*	1923, CLELAND 1924, EMERSON 1926, CLELAND 1928, CLELAND 1931, LELIVELD 1931, DARLINGTON	DAVIS (DE VRIES) DAVIS (?) DAVIS (DE VRIES) RENNER (R-*biennis*) STOMPS Seed growing wild at Camberley	Wild species; said by BOEDIJN to be derived from *lamarckiana;* gives unlike reciprocal hybrids (egg and sperm lethals); intermediate flower size; permanently heterozygous (CLELAND); STOMPS does not consider it a hybrid and thinks it has been for centuries a constant, pure, and truly uniform species. Complex *rubens.albicans*. Also shows ⊙ 14; or ⊙ 4 and loose chromosomes; or 3 pairs in ovules.
biennis sulfurea*	1923, CLELAND 1925, EMERSON 1926, CLELAND	DAVIS (DE VRIES) DAVIS (DE VRIES) DAVIS (DE VRIES)	Mutant of *biennis;* also shows ⊙ 14.
biennis cruciata	1932, ILLICK	SHULL	Mutant from *biennis* by STOMPS; differs from wild *biennis* in having narrow petals.
novae-scotiae*	1927, SHEFFIELD	GATES	Segregate from *muricata* which latter has bred true for many years; (*muricata* possibly from *biennis*); some non-disjunction; small flowers. Also shows ⊙ 14 as more common configuration.
rubricalyx,* pure	1929, RUDLOFF	?	See table II. Also shows 4 pairs and ⊙ 6; or 5 pairs and ⊙ 4.

* Indicates variable configuration of chromosomes.

of closed circles and permits the assumption of specific genes as causal factors.

Table VIII (⊙s 4 and 10) consists of only one form, *aurata*, a segregate from *franciscana*×*biennis*, with *lamarckiana* in its ancestry.

Table IX (⊙ 14) includes thirty individuals which consist of twenty species, seven mutants, one hybrid derivative, and two seg-

regates. Thirteen of the thirty were not mentioned in the preceding tables and have shown only one type of configuration. The other seventeen have shown various types of chromosomal configurations other than the ⊙ 14, and have been previously mentioned in at least one of the other eight tables.

Information is available for fifteen of these thirty forms which shows that the configurations of known offspring are identical to those of the parents concerned. *Pervirens*, a mutant from *lamarckiana*, is known to have shown three of the configurations seen in *lamarckiana*. *Biennis* has thrown *biennis sulfurea* as a mutant and possibly *muricata*. The latter in turn has segregated *novae-scotiae*. These all have one configuration in common and in some cases two.

TABLE VIII

CHROMOSOME CONFIGURATION WITH ⊙S 4 AND 10

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
aurata*	1927, CLELAND	SHULL	See table II. Also shows 5 pairs and ⊙ 4; or 1 pair and ⊙ 12.

* Indicates variable configuration of chromosomes.

Suaveolens (⊙ 14) and *strigosa* (⊙ 14), two different species, when crossed, give *albata*, which also shows the same configuration. *Pratincola* has six mutants in this table, all of which show the same configuration, ⊙ 14.

It is to be noticed that in the case of *biennis* L. some investigators emphasize it as being permanently heterozygous, while by others it is said to be "a constant, pure, and uniform species."

While some of these forms showing ⊙ 14 are known to be stable genetically, as *muricata* and *grandiflora*, they are also known to have shown various types of chromosomal configurations. They both have shown three types of configurations, ⊙ 14, chain of 12 and 1 pair, chain of 10 and 2 pairs and ⊙ 14, ⊙ 5 and 4 pairs, 7 pairs, respectively.

There is also *grandiflora* Ait., a "complex heterozygote," with three types of configurations, and showing segregation in each generation of two forms (the third being non-viable), apparently without regard to the type of configuration possessed.

TABLE IX

CHROMOSOME CONFIGURATION WITH ⊙ 14

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
lamarckiana*	1931, LELIVELD	STOMPS	See table I. Also shows ⊙ 12 and 1 pair; or ⊙ 10 and 2 pairs; or 3 pairs and chain; or 7 pairs. Configuration here is chain of 14.
pervirens*	1929, ILLICK 1932, ILLICK	SHULL SHULL	See table I. Also shows ⊙ 12 and 1 pair as more common condition; or 7 pairs. Plant of 1932 was a selfed individual and showed ⊙ 14, and ⊙ 12 and 1 pair in cells four sections apart.
biennis L.*	1923, CLELAND 1931, DARLINGTON	DAVIS (DE VRIES) From seeds growing wild at Camberley	See table VII. Also shows ⊙s 6 and 8; or ⊙ 4 and loose chromosomes; or 3 pairs and loose chromosomes in ovules.
biennis sulfurea*	1923, CLELAND	DAVIS (DE VRIES)	See table VII. Also shows ⊙s 6 and 8.
suaveolens* (yellow)	1926, OEHLKERS	OEHLKERS	See table VI. Also shows ⊙ 12 and 1 pair.
albata*	1929, OEHLKERS	OEHLKERS	See table VI. Also shows ⊙ 12 and 1 pair.
pratincola,* Bartlett	1929, KULKARNI	DAVIS	See table VI. Also shows ⊙ 12 and 1 pair.
simulans*	1929, "	"	See table VI. Also shows ⊙ 12 and 1 pair.
recidiva	1929, "	"	Mutant of *pratincola* (Bartlett); carried as selfed line since 1915 and gives two types in every generation, itself (⊙ 14) and stunted non-flowering plant (7 pairs).
formosa	1929, "	"	Mutant of *pratincola* (Bartlett).
pratincola C.	1929, "	"	Strain of *pratincola* (Bartlett).
pratincola E.	1929, "	"	Strain of *pratincola* (Bartlett).
pratincola M.	1929, "	"	From *formosa*×strain C.

* Indicates variable configuration of chromosomes.

TABLE IX—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
muricata*	1923, CLELAND	DAVIS (DE VRIES)	See table V. Also shows 1 pair and chain of 12; or 2 pairs and chain of 10.
	1926, CLELAND	DAVIS (DE VRIES)	
	1931, LELIVELD	STOMPS	
novae-scotiae*	1927, SHEFFIELD	GATES	See table VII. Also shows ⊙s 6 and 8 as less common condition.
cockerelli*	1926, OEHLKERS	OEHLKERS	See table VI. Also shows ⊙ 12 and 1 pair as less common condition.
	1929, CLELAND and OEHLKERS	OEHLKERS	
strigosa*	1926, OEHLKERS	OEHLKERS	See table VI. Also shows ⊙ 12 and 1 pair as less common condition.
ammophila*	1927, SHEFFIELD	GATES (RENNER)	See table VI. Also shows ⊙ 12 and 1 pair as less common condition.
agari*	1927, "	GATES	Small flowers; sterile pollen and nonviable seeds relatively low; very uniform in appearance and behavior except for occurrence of one narrow leaved mutant; no cases of non-disjunction found; will cross with species of the *lamarckiana* group, while within this group the species can be readily intercrossed, although in nature many of them are self-pollinated (**28**). Also shows variable chromosome conditions including a ⊙ 3.
eriensis	1927, "	"	Wild species; small flowers; sometimes non-disjunction; plants remarkably uniform in appearance; collections from six different plants along Lake Erie; so similar to *ammophila* that it might be considered a subspecies.
angustissima	1929, GATES and SHEFFIELD	GATES (SHARP)	Wild species; small flowers; non-disjunction and double non-disjunction; considered a permanent hybrid as other small flowered species (**28**).
sinuata L.	1927, SINOTO	?	"A species belonging to the Eu-*Oenothera* group" (**46**).
pycnocarpa	1930, CATCHESIDE	GATES	Wild species; small flowers; permanent hybrid; closely related to *nutans*.

* Indicates variable configuration of chromosomes.

TABLE IX—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
nutans	1930, CATCHESIDE	GATES	Wild species; small flowers; permanent hybrid.
germanica*	1931, LELIVELD	STOMPS	See table IV. Also shows ⊙ 12 and 1 pair; or ⊙ 10 and 2 pairs; or 3 pairs and loose chromosomes.
grandiflora B*	1929, CLELAND and OEHLKERS	DEVRIES	See table I. Also shows 7 pairs; or ⊙ 4 and 5 pairs.
grandiflora* Ait.	1929, GERHARD	DEVRIES	See table I. Also shows ⊙ 12 and 1 pair; or 7 pairs.
pachycarpa	1930, RUDLOFF	?	Species with complex *augens.subcurvans*.
rosea	1924, SCHWEMMLE	?	Subgenus *hartmannia*.
berteriana	1931, DARLINGTON	RENNER	Species.

* Indicates variable configuration of chromosomes.

The evidence here presented, some of which was mentioned in previous tables, certainly indicates that neither homozygosity nor heterozygosity has any direct bearing on the formation of closed circles. When a careful study is made of the parents and their offspring mentioned in this table they invariably have been shown to possess at least one and possibly several chromosomal configurations in common, which permits the assumption that closed circles are characters, as are the usual phenotypical characters, and as such, the expression of factor activity.

Table X consists of five trisomic individuals none of which was mentioned in the preceding tables. Two, *oblonga* and *pulla*, have shown more than one type of configuration. *Oblonga* is a trisomic mutant from *lamarckiana* or from *scintillans* selfed, and *pulla* is also a trisomic mutant from *lamarckiana*. Of the other three, *lata* and *stricta* (the former a trisomic mutant from *lamarckiana*) have shown the same type of configuration, ⊙ 13 and 1 pair, while *scintillans* shows 7 complete pairs and one unpaired chromosome.

Table XI includes only those individuals which have shown vari-

able chromosomal configurations, of which there are at present twenty-nine. Since these have already been discussed in one or more

TABLE X

TRISOMICS

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
lata	1928, HÅKANSSON	?	Trisomic mutant of *lamarckiana;* first discovered by DEVRIES in 1887; almost completely male sterile; forms chromosome fragments in meiotic division of pollen mother cells. "Wherever extra chromosome is present, splitting will occur in offspring no matter with what other features the *lata* characters may be associated" (**26**). HÅKANSSON studied ovules. Configuration is ⊙ 13 and 1 pair.
stricta	1928, HÅKANSSON	?	Shows ⊙ 13 and 1 pair.
scintillans	1918, HANCE	DEVRIES	Has appeared only rarely; derived from *lata* and *lamarckiana.* When selfed "it regularly produces *lamarckiana, oblonga* and *scintillans* in varying proportions together with occasional mutants such as *lata* and *nanella*" (GATES). Shows 7 pairs and 1 unpaired.
oblonga*	1923, CLELAND	DAVIS	Trisomic mutant from *lamarckiana* by DEVRIES, may also arise from *scintillans* selfed. Shows ⊙ 5 and 5 pairs; or ⊙ 3; or chains of 5, 7, 9 and others paired.
pulla*	1928, HÅKANSSON	?	Trisomic mutant from *lamarckiana;* in heterotypic anaphase the distribution in trisomics is 7 and 8 (HÅKANSSON). Shows mostly ⊙ 6, 3 pairs and 1 trivalent; other conditions seen but not definitely stated; ovules studied.

* Indicates variable configuration of chromosomes.

of the preceding tables, nothing more need be said at this time except to emphasize (1) the difficulty of assuming that chromosomal configurations are constant for any one species; and (2) that whenever such various configurations have occurred, there were observed no corresponding phenotypical changes which might warrant the con-

TABLE XI

VARIABLE CHROMOSOME CONFIGURATIONS

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
lamarckiana (velans. gaudens)	1924, BOEDIJN	Original strain of DEVRIES	7 pairs (?)	cf tables I, IV, V, VI, IX.
	1925, CLELAND	SHULL (DEVRIES)	⊙ 12 and 1 pair	
	1926, HÅKANSSON	?	⊙ 12 and 1 pair	
	1928, HÅKANSSON	HERIBERT-NILSSON	⊙ 12 and 1 pair	
	1929, CLELAND	RENNER (r-*lamarckiana*)	⊙ 12 and 1 pair	
	1929, CLELAND	DEVRIES	⊙ 12 and 1 pair	
	1929, ILLICK	SHULL (DEVRIES)	⊙ 12 and 1 pair	
	1931, LELIVELD	STOMPS	⊙ 12 and 1 pair; ⊙ 10 and 2 pairs; chain and 3 pairs; chain of 14; and all or almost all loose	
pervirens mut. (velans. gaudens)?	1929, ILLICK	SHULL	⊙ 12 and 1 pair; in another pedigreed plant 7 pairs; in selfed plant ⊙ 14, and ⊙ 12 and 1 pair were seen in same bud four sections apart	cf tables I, VI, IX. ⊙ 12 and 1 pair most common.
stenophylla (selfed)	1932, ILLICK	SHULL	⊙ 12 and 1 pair; ⊙ 6 and 4 pairs, the latter seen in two plants of same pedigree	cf tables III, VI. ⊙ 6 and 4 pairs more common.
oblonga	1923, CLELAND	DAVIS	⊙ 5 and 5 pairs; ⊙ 3; chains of 5, 7, 9, and others paired	cf table X.
pulla	1928, HÅKANSSON	?	Mostly ⊙ 6, 3 pairs and 1 trivalent	cf table X. Also other conditions but not definitely stated.
cockerelli (elongans. curtans)	1926, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX. ⊙ 14 more common.

TABLE XI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
biennis L. (rubens. albicans)	1923, CLELAND	DAVIS (DEVRIES)	⊙ 14; ⊙s 6 and 8	cf tables VII, IX. ⊙s 6, 8 more common.
	1925, EMERSON	DAVIS (DEVRIES)	⊙s 6 and 8	
	1926, CLELAND	DAVIS (DEVRIES)	⊙s 6 and 8	
	1928, CLELAND	RENNER (R-*biennis*)	⊙s 6 and 8	
	1931, LELIVELD	STOMPS	⊙6 s and 8; ⊙ 4 and loose chromosomes; in ovules 3 pairs and loose chromosomes	
	1931, DARLINGTON	Seed growing wild at Camberley	⊙ 14 in plant A and C; ⊙s 6 and 8 plant B	
biennis sulfurea	1923, CLELAND	DAVIS (DEVRIES)	⊙ 14; ⊙s 6 and 8	cf tables VII, IX. ⊙s 6 and 8 more common.
	1925, EMERSON	DAVIS (DEVRIES)	⊙s 6 and 8	
	1926, CLELAND	DAVIS (DEVRIES)	⊙s 6 and 8	
biennis gigas	1931, LELIVELD	STOMPS	Several pairs and loose chromosomes; or chromosomes linked and no pairs; or all chromosomes detached; nothing more definite given	From *biennis* × *biennis cruciata* by STOMPS, 1919.
	1929, HOEPPENER and RENNER	RENNER	Several pairs	
suaveolens (yellow) (flavens.albicans)	1926, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
	1928, CLELAND	RENNER (DEVRIES)	⊙ 12 and 1 pair	
	1929, ILLICK	SHULL	⊙ 12 and 1 pair	
strigosa (deprimens. stringens)	1926, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
albata	1926, 1929, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
flava	1926, 1929, OEHLKERS	OEHLKERS	7 pairs; ⊙ 4 and 5 pairs	cf tables I, II. F_1 seg. of *suaveolens* × *strigosa* as is also *albata* above.

TABLE XI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
pratincola, Bartlett	1929, KULKARNI	DAVIS	⊙ 14	cf tables VI, IX.
	1929, ILLICK	SHULL	⊙ 12 and 1 pair	
simulans	1929, KULKARNI	DAVIS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
muricata (curvans. rigens)	1923, CLELAND	DAVIS (DEVRIES)	⊙ 14	cf tables V, VI, IX.
	1926, CLELAND	DAVIS (DEVRIES)	⊙ 14	
	1931, LELIVELD	STOMPS	⊙ 14; chain 12 and 1 pair; chain 10 and 2 pairs; and loose or almost loose chromosomes	
novae-scotiae	1927, SHEFFIELD	GATES	⊙ 14; ⊙s 6 and 8	cf tables VII, IX. ⊙ 14 more common.
agari	1927, SHEFFIELD	GATES	⊙ 14; or variety of conditions including ⊙ 3	cf table IX. ⊙ 14 very common.
ammophila (rigens. percurvans)	1927, SHEFFIELD	GATES (RENNER)	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
	1932, ILLICK	SHULL	⊙ 12 and 1 pair	
rubrinervis (laeta. velutina)	1908, GATES	GATES	⊙ 6 and 4 pairs; 7 pairs	cf tables I, III.
	1925, CLELAND	SHULL	⊙ 6 and 4 pairs (one plant)	
	1928, CLELAND	SHULL (DAVIS)	⊙ 6 and 4 pairs	
	1928, HÅKANSSON	?	⊙ 6 and 4 pairs in ovules	
	1929, ILLICK	SHULL	⊙ 6 and 4 pairs	
rubricalyx, pure (rubens. tingens)	1927, SHEFFIELD	GATES	⊙ 4 and 5 pairs; ⊙ 6 and 4 pairs	cf tables II, III, VII. Variables also seen.
	1929, GATES and SHEFFIELD	GATES	⊙ 4 and 5 pairs; ⊙ 6 and 4 pairs	
	1929, RUDLOFF	?	⊙ 6 and 8	
"heterozygous for 7 characters"	1928, CLELAND	SHULL	⊙ 12 and 1 pair; ⊙ 10 and 2 pairs	cf tables V, VI.
rubrisepala	1928, 1930, HÅKANSSON	?	⊙ 6 and 4 pairs ⊙ 4 and 5 pairs	cf tables II, III.

TABLE XI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
germanica	1931, LELIVELD	STOMPS	⊙ 14; ⊙ 12 and 1 pair; ⊙ 10 and 2 pairs (these seen in one and same slide); 3 pairs and loose chains; or all detached	cf tables IV, V, VI, IX.
grandiflora B (truncans. acuens)	1909, DAVIS	DAVIS	7 pairs	cf tables I, II, IX.
	1910, CLELAND	SHULL	⊙ 4 and 5 pairs	
	1928, CLELAND	SHULL (DAVIS)	7 pairs	
	1929, ILLICK	SHULL (DAVIS)	7 pairs	
	1929, CLELAND and OEHLKERS	DEVRIES	⊙ 14	
grandiflora Ait. (truncans.acuens)	1929, GERHARD	?	⊙ 14; ⊙ 12 and 1 pair; 7 pairs	cf tables I, VI, IX.
franciscana	1922, CLELAND	DAVIS (BARTLETT)	⊙ 4 and 5 pairs	cf tables I, II.
	1928, CLELAND	SHULL (DAVIS)	7 pairs	
	1928, KULKARNI	DAVIS (BARTLETT)	⊙ 4 and 5 pairs; 7 pairs	
	1929, HOEPPENER and RENNER	?	7 pairs	
	1931, LELIVELD	STOMPS	7 pairs; chain of 4 or more	
franciscana sulfurea	1923, 1924, CLELAND	DAVIS	⊙ 12 and 1 pair	cf tables I, VI.
	1928, CLELAND	SHULL (DAVIS)	7 pairs	
	1929, ILLICK	SHULL (DAVIS)	7 pairs	
aurata	1927, CLELAND	SHULL	⊙s 4 and 10	cf tables II, VI, VIII.
	1928, CLELAND	SHULL	⊙ 4 and 5 pairs; ⊙ 12 and 1 pair	
	1932, ILLICK	SHULL	⊙ 4 and 5 pairs	

clusion that changes in the formation of closed circles are in any way associated with the appearance of the plant. The two forms, *per-*

virens and *germanica*, each of which has shown in one slide two or more types of configurations, would be especially difficult to explain on this hypothesis.

The members of this table of variables consist of eleven species, nine mutants and half-mutants, five segregates, one hybrid derivative, and three of unknown make-up. It appears, therefore, that the condition governing variable configurations is not restricted to any one genetical type of *Oenothera*.

In view of the data presented thus far, it would be more consistent to assume that closed circles are characters as are the generally recognized phenotypical characters of the Oenotheras and as such, the expression of heritable factors.

Table XII consists of F_1 and F_2 individuals of 105 *Oenothera* crosses. Some of these crosses are duplicates in that the parents are the same in name, but the genetical complex in the parents has been recorded as being different.

By carefully checking the chromosomal configurations of the hybrids with those of their parents, it will be found that sixty-four show common configurations in this respect while forty-one have so far shown no resemblance in configurations between the two parents, or the one known parent, and the offspring. Two of these forty-one were trisomics and two had only one parent whose configuration was known. Further study of the thirty-nine diploid individuals may later reveal in some instances configurations which resemble those of one or both parents. Such a condition is not necessary, however, since in certain cases it might be expected that the chromosomal configuration of the offspring would not conform to those of the parents owing to the failure to inherit the proper genic combination.

In one instance the F_1 of (*biennis*×*rubricalyx*)×*ammophila*, a triple heterozygote, showed only 7 complete pairs which GATES and SHEFFIELD (**34**) claim "makes it clear that complete pairing cannot be regarded as a sign of the homozygous condition." Twelve other triple hybrids in this table have also shown 7 complete pairs of chromosomes. Hybridity, as such, apparently acts as no deterrent to complete pairing at diakinesis.

TABLE XII

CHROMOSOME CONFIGURATIONS OF OENOTHERA CROSSES

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
seg. decipiens ×grandiflora B	1929, ILLICK	SHULL	⊙ 6 and 4 pairs	♀ stable; no lethals. ♂ 7 pairs; ⊙ 4 and 5 pairs; ⊙ 14. cf tables I, II, IX, XI.
(seg. decipiens ×grandiflora B) F_2	1929, ILLICK	SHULL	7 pairs common; ⊙ 6 and 4 pairs	♀ ♂ cf above. Hybrid showing 7 pairs.
(grandiflora B ×seg. decipiens) F_2	1929, ILLICK	SHULL	7 pairs common; ⊙ 6 and 4 pairs	♀ ♂ cf above. Hybrid showing 7 pairs
grandiflora B ×hookeri	1929, CLELAND and OEHLKERS	OEHLKERS	⊙ 14	♀ cf above. ♂ 7 pairs. cf table I. Complex *truncans.*^{h}*hookeri;* no factor exchange except flower size.
grandiflora B ×hookeri, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	2 ⊙s 4 and 3 pairs	♀ ♂ cf above. Complex *acuens.*^{h}*hookeri* and recip. At least four independent factor exchanges between complexes.
grandiflora Ait. ×hookeri	1929, GERHARD	?	⊙ 8 and 3 pairs	♀ 7 pairs; ⊙ 14; ⊙ 12 and 1 pair. cf tables I, VI, IX, XI. ♂ cf above.
grandiflora B ×lamarckiana, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	⊙ 14	♀ cf above. ♂ 7 pairs (?); 3 pairs and chain; 2 pairs and ⊙ 10; 1 pair and ⊙ 12; chain of 14. cf tables I, IV, V, VI, IX, XI. Complex *acuens.gaudens;* no factor exchange except flower size.
grandiflora B ×lamarckiana, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	⊙s 10 and 4	♀ ♂ cf above. Complex *truncans.velans* and recip. and *truncans.gaudens* and recip. No factor exchange except flower size.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
grandiflora B ×lamarckiana, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	⊙s 6 and 4 and 2 pairs	♀ ♂ cf above. Complex *acuens.velans;* considerable factor exchange.
grandiflora Ait.×lamarckiana	1929, GERHARD	?	⊙s 6 and 4 and 2 pairs	♀ ♂ cf above. Complex *acuens.velans.*
lamarckiana ×grandiflora Ait.	1929, "	?	7 pairs	♀ ♂ cf above. Complex *truncans.velans.*
lamarckiana ×grandiflora Ait.	1929, "	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex *acuens.gaudens.*
(lamarckiana ×grandiflora Ait.) F_2	1929, "	?	⊙ 4 and 5 pairs	♀ ♂ cf above. Complex *acuens.velans.*
grandiflora B ×franciscana	1928, CLELAND	SHULL	⊙ 4 and 5 pairs	♀ cf above. ♂ 7 pairs; 5 pairs and ⊙ 4; chain of 4 or more. cf tables I, II, XI.
grandiflora B ×franciscana	1928, EMERSON	?	Configuration resembles one or other parent	♀ ♂ cf above.
franciscana× grandiflora B	1928, CLELAND	SHULL	5 pairs and ⊙ 4	♀ ♂ cf above.
franciscana× grandiflora B	1928, EMERSON	?	Resembles one or other parent	♀ ♂ cf above.
"mut. sulfurea"×grandiflora B	1928, CLELAND	SHULL	⊙ 6 and 4 pairs; 7 pairs	♀ ⊙ 6 and 4 pairs. cf table III. ♂ cf above. Hybrid with 7 pairs.
grandiflora B ×"mut. sulfurea"	1928, CLELAND	SHULL	⊙ 6 and 4 pairs; 7 pairs	♀ ♂ cf above. Hybrid with 7 pairs.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
grandiflora Ait. × suaveolens	1929, GERHARD	?	⊙ 4 and 5 pairs	♀ cf above. ♂ ⊙ 14; ⊙ 12 and 1 cf tables VI, IX, XI. Complex *flavens.acuens.*
grandiflora Ait. × suaveolens	1929, GERHARD	?	⊙ 14	♀ ♂ cf above. Complex *flavens.truncans.*
grandiflora Ait. × muricata	1929, GERHARD	?	⊙ 10 and 2 pairs	♀ cf above. ♂ ⊙ 14; chain 12 and 1 pair; chain 10 and 2 pairs; loose or almost loose chromosomes. cf tables V, VI, IX, XI. Complex *curvans.truncans.*
muricata × grandiflora Ait.	1929, GERHARD	?	⊙s 8, 4 and 1 pair	♀ ♂ cf above. Complex *rigens.acuens.*
muricata × grandiflora Ait.	1929, GERHARD	?	⊙ 14	♀ ♂ cf above. Complex *rigens.truncans.*
grandiflora Ait. × biennis cruciata	1929, GERHARD	?	⊙s 10, 4	♀ cf above. ♂ ⊙s 6, 8. cf table VII. Complex *rubens.acuens.*
grandiflora Ait. × biennis cruciata	1929, GERHARD	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex *albicans.acuens.*
grandiflora Ait. × cruciata	1929, GERHARD	?	⊙s 10, 4	♀ cf above. ♂ ? Complex *truncans.flectens.*
cockerelli × grandiflora Ait.	1929, GERHARD	?	⊙ 14	♀ ⊙ 14; ⊙ 12 and 1 pair. cf tables VI, IX, XI. ♂ cf above. Complex *curtans.truncans.*
grandiflora Ait. × biennis cruciata	1929, GERHARD	?	⊙ 12 and 1 pair	♀ ♂ cf above. Complex *albicans.truncans.*
grandiflora Ait. × biennis cruciata	1929, GERHARD	?	⊙ 14	♀ ♂ cf above. Complex *rubens.truncans.*

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
franciscana sulfurea × latifrons	1928, CLELAND	SHULL	⊙ 6 and 4 pairs	♀ 7 pairs common; 1 pair and ⊙ 12. cf tables I, VI, XI. ♂ 7 pairs. cf table I.
aurata × latifrons	1928, CLELAND	SHULL	⊙ 4 and 5 pairs; ⊙ 6 and 4 pairs	♀ ⊙ 4 and 5 pairs; ⊙ 12 and 1 pair; ⊙s 10, 4. cf tables II, VI, VIII, XI. ♂ cf above.
aurata × bullata	1932, ILLICK	SHULL	⊙ 6 and 4 pairs	♀ cf above. ♂ ⊙ 12 and 1 pair. cf table VI.
rubricalyx × grandiflora B	1925, CLELAND	?	⊙ 8 and 3 pairs	♀ ⊙ 6 and 4 pairs; ⊙ 4 and 5 pairs. cf tables II, III, XI. ♂ cf above.
rubricalyx × hewettii	1923, GATES	GATES	⊙ 5 and 5 pairs	♀ cf above. ♂ Chromosomes may be like *franciscana;* did not breed true in GATES's cultures. Both parents distinct species. Mutant of this cross unnamed.
hewettii × rubricalyx	1923, GATES	GATES	⊙ 5 and 5 pairs	♀ ♂ cf above.
ammophila × (biennis × rubricalyx) F_2	1929, GATES and SHEFFIELD	GATES	⊙ 8 and 3 pairs; ⊙ 4 and 5 pairs or ⊙ 12 and 1 pair	♀ ⊙ 12 and 1 pair; ⊙ 14. cf tables VI, IX, XI. ♂ *Biennis* shows ⊙s 6, 8; or ⊙ 14; or ⊙ 4 and loose chromosomes; or 3 pairs and loose chromosomes. *Rubricalyx* shows ⊙ 6 and 4 pairs; or ⊙ 4 and 5 pairs. cf tables II, III, VII, IX, XI. Triple heterozygote, breeds true except for minor features as bud color. In this hybrid "the amount of somatic pairing appears to be variable in the individual and independent of the arrangement taken up during meiosis."

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(biennis×rubricalyx)×ammophila	1929, GATES and SHEFFIELD	GATES	7 pairs	♀ ♂ cf above. "This is the first case in which unlike linkages have been recorded in reciprocal hybrids"; triple hybrid with 7 pairs "makes it clear that complete pairing cannot be regarded as a sign of the homozygous condition."
ammophila×rubricalyx der.	1929, SHEFFIELD	?	⊙ 6 and 4 pairs	♀ ♂ cf above.
lamarckiana×suaveolens sulfurea, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	⊙ 12 and 1 pair	♀ cf above. ♂ ⊙ 12 and 1 pair. cf table VI. Complex *gaudens.flavens*. Occasional transfer of *flavens* lethal.
lamarckiana×suaveolens sulfurea	1929, "	"	2 ⊙s 4 and 3 pairs	♀ ♂ cf above. Complex *velans.flavens* and recip. Exchange of at least three independent factors.
suaveolens sulfurea×lamarckiana	1929, "	"	⊙ 14	♀ ♂ cf above. Complex *albicans.velans*. No factor exchange except flower size.
suaveolens sulfurea×lamarckiana	1929, "	"	⊙s 6, 8	♀ ♂ cf above. Complex *albicans.gaudens*. Factor exchange only in flower size.
lamarckiana cruciata×strigosa	1929, "	"	⊙s 6, 4, and 2 pairs	♀ ⊙ 12 and 1 pair. cf table VI. ♂ ⊙ 14; ⊙ 12 and 1 pair. cf tables VI, IX, XI. Complex *velans.stringens*. Considerable factor exchange.
lamarckiana cruciata×strigosa	1929, "	"	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex *deprimens.velans* and *deprimens.gaudens*. Occasional split in leaf breadth.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
lamarckiana cruciata× strigosa	1929, CLELAND and OEHLKERS	OEHLKERS	⊙ 14	♀ ♂ cf above. Complex *gaudens.stringens.*
suaveolens sulfurea× strigosa	1929, "	"	⊙ 12 and 1 pair	♀ ♂ cf above. Complex *albicans.stringens.* Exchange only in pollen sterility factor.
suaveolens sulfurea× strigosa	1929, "	"	⊙ 12 and 1 pair	♀ ♂ cf above. Complex *deprimens.flavens.* No factor exchange except in flower size.
suaveolens sulfurea× strigosa	1929, "	"	⊙ 4 and 5 pairs	♀ ♂ cf above. Complex *flavens.stringens.* Exchange in at least four independent factors.
suaveolens× cockerelli, and recip.	1929, "	"	⊙ 12 and 1 pair	♀ ♂ cf above. Complex *albicans.elongans.* No factor exchange according to DEVRIES.
suaveolens× cockerelli, and recip.	1929, "	"	⊙ 12 and 1 pair	♀ ♂ cf above. Complex *curtans.flavens.* No factor exchange.
suaveolens× cockerelli, and recip.	1929, "	"	⊙ 8 and 3 pairs	♀ ♂ cf above. Complex *flavens.elongans.* Occasional factor exchange of four factors.
suaveolens× strigosa	1926, OEHLKERS	"	⊙ 12 and 1 pair; 7 pairs	♀ ♂ cf above. Complex *flavens.stringens.* F_1 gave twin hybrids, *albata* and *flava*, former with ⊙ 12 and 1 pair breeding true and latter with 7 pairs segregating many types.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
suaveolens × strigosa	1929, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 4 and 5 pairs	♀ ♂ cf above. F_1 gave twin hybrids, *albata* and *flava;* former is constant in breeding behavior (⊙ 14; ⊙ 12 and 1 pair); latter segregates many forms (⊙ 4 and 5 pairs; 7 pairs).
biennis × suaveolens	1928, CLELAND 1929, HOEPPENER and RENNER	DEVRIES ?	⊙ 12 and 1 pair	♀ ⊙s 6 and 8; ⊙ 14; ⊙ 4 and loose chromosomes; 3 pairs and loose chromosomes. cf table VII, IX, XI. ♂ cf above. "Hybrid is in reality practically or wholly identical with the species *suaveolens* and indistinguishable from it phenotypically. When selfed, it breeds true for this configuration" (**14**). Complex *albicans.flavens.*
(suaveolens × pachycarpa) F_2	1930, RUDLOFF	?	⊙ 6 and 4 pairs; ⊙s 6 and 4 and 2 pairs	♀ cf above. ♂ ⊙ 14. cf table IX.
suaveolens × pachycarpa	1930, "	?	⊙ 6 and 2 ⊙s 4	♀ ♂ cf above. Complex *flavens.subcurvans.*
muricata × pachycarpa	1930, "	?	⊙ 14	♀ ♂ cf above. Complex *rigens.subcurvans.*
lamarckiana × pachycarpa	1930, "	?	⊙ 14	♀ ♂ cf above. Complex *subcurvans.velans.*
pachycarpa × lamarckiana	1930, "	?	⊙ 12 and 1 pair	♀ ♂ cf above. Complex *augens.velans.*
(purpurata × suaveolens) F_2	1929, "	?	7 pairs	♀ 7 pairs. cf table I. ♂ cf above. Complex *hpurpurata.flavens.*

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(purpurata × suaveolens) × biennis	1929, RUDLOFF	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex *rubens.flavens.*
purpurata × cruciata	1929, "	?	⊙ 4 and 5 pairs	♀ cf above. ♂ ? Complex *hpurpurata. flectens.*
(purpurata × lamarckiana) F_2	1929, "	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex *hpurpurata. gaudens.*
(purpurata × lamarckiana) × purpurata	1929, "	?	⊙ 12 and 1 pair	♀ ♂ cf above. Complex *hpurpurata. velans.*
hookeri × lamarckiana	1929, HOEPPENER and RENNER	RENNER	⊙ 4 and 5 pairs; 3 pairs and 2 ⊙s 4	♀ 7 pairs. cf table I. ♂ cf above. Complex *hhookeri.velutina.*
hookeri × lamarckiana	1929, HOEPPENER and RENNER	RENNER	⊙ 10 and 2 pairs (probably)	♀ ♂ cf above. Complex *hhookeri.gaudens.*
hookeri × suaveolens	1928, CLELAND 1930, CLELAND and BLAKESLEE	DEVRIES	⊙ 4 and 5 pairs	♀ ♂ cf above. Complex *flavens.hhookeri.*
suaveolens × hookeri	1930, CLELAND and BLAKESLEE	?	⊙ 14	♀ ♂ cf above. Complex *albicans. hhookeri.*
hookeri × biennis	1930, CLELAND and BLAKESLEE	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex *hhookeri.rubens.*
biennis × hookeri	1928, CLELAND	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex *rubens.hhookeri.*
R-biennis × hookeri	1928, CLELAND 1929, HOEPPENER and RENNER	RENNER RENNER	⊙ 14 common (*albata*); ⊙ 10 and 2 pairs (*rubefacta*)	♀ ⊙s 6 and 8. cf table VII. ♂ cf above. Complex *albicans. hhookeri.*

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
chicagoensis ×hookeri	1930, CLELAND and BLAKESLEE	CLELAND and BLAKESLEE	⊙ 4 and 5 pairs	♀ ⊙ 12 and 1 pair. cf table VI. ♂ cf above. Complex *excellens.* ^{h}*hookeri.*
chicagoensis ×biennis	1931, CLELAND	?	⊙s 6, 8	♀ ♂ cf above. Complex *punctulans. rubens;* metacline hybrid.
biennis×metacline	1931, CLELAND	?	⊙s 6, 8	♀ cf above. ♂ ⊙s 6, 8. cf table VII. Complex *albicans.rubens.*
chicagoensis ×suaveolens	1930, CLELAND and BLAKESLEE	CLELAND and BLAKESLEE	2 ⊙ 4 and 3 pairs	♀ ♂ cf above. Complex *excellens.flavens.*
lamarckiana ×chicagoensis	1930, CLELAND and BLAKESLEE	CLELAND and BLAKESLEE	⊙ 6 and 4 pairs	♀ ♂ cf above. Complex *velans.excellens.*
pachycarpa × hookeri	1930, RUDLOFF	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex ^{h}*hookeri.augens.*
biennis× pachycarpa	1930, RUDLOFF	?	⊙ 14	♀ ♂ cf above. Complex *albicans.subcurvans.*
biennis×muricata	1928, CLELAND	DEVRIES	⊙s 4, 6 and 2 pairs; ⊙ 4 and 5 pairs	♀ ♂ cf above.
biennis×lamarckiana	1930, HÅKANSSON	?	⊙s 6, 8	♀ ♂ cf above. Complex *albicans.gaudens*(?).
biennis×lamarckiana	1930, HÅKANSSON	?	⊙ 14	♀ ♂ cf above. Complex *albicans.velans.*
biennis sulfurea, hannover×cockerelli	1929, CLELAND and OEHLKERS	OEHLKERS	⊙ 12 and 1 pair	♀ ? ♂ cf above. Complex *albicans.elongans.* No exchange except in flower size, to which one or more genes are linked.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
lamarckiana ×rubricalyx, Afterglow	1930, EMERSON	?	⊙ 12 and 1 pair	♀ cf above. ♂ ⊙ 8 and 3 pairs. cf table IV. Complex *gaudens*. modified *velans* of *rubricalyx*. F_1 has *lamarckiana* growth habits.
lamarckiana ×biennis, Chicago	1928, CLELAND 1928, HÅKANSSON	deVRIES ?	⊙ 12 and 1 pair	♀ cf above. ♂ ? Heterogamous (pollen and egg carry different qualities). This cross gives *fallax* (table VI).
(lamarckiana ×rubricalyx, Afterglow)F_1 (selfed)	1930, EMERSON	?	⊙ 12 and 1 pair	♀ ♂ cf above. F_1 of above cross selfed. Breeds true for bud color, growth habit, and chromosome configuration.
lamarckiana ×rubricalyx, Afterglow	1930, "	?	⊙ 8 and 3 pairs	♀ ♂ cf above. Complex *gaudens.latifrons* of *rubricalyx* (no lethal). F_1 of this cross resembles *lamarckiana* in growth habit.
(lamarckiana ×rubricalyx, Afterglow)F_1 (selfed)	1930, "	?	7 pairs	♀ ♂ cf above. F_1 of above cross selfed. Gives two types: (1)F_1 type with growth habits variable, and (2) type homozygous for *rubricalyx* bud color, bud shape of mut. *latifrons*, and growth habit variable. Hybrid with 7 pairs.
lamarckiana ×rubricalyx, Afterglow	1930, "	?	⊙ 8 and 3 pairs	♀ ♂ cf above. Complex *velans.latifrons*. F_1 has *rubricalyx* growth habit.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(lamarckiana × rubricalyx, Afterglow) F_1 (selfed)	1930, EMERSON	?	⊙ 8 and 3 pairs; 7 pairs	♀ ♂ cf above. F_1 of above cross selfed. First configuration seen in two types: *rubricalyx* habits and buds and *rubricalyx* habits and red buds; second configuration seen in two types: *latifrons* habits and *rubricalyx* buds, and *latifrons* habits and red buds. Hybrid with 7 pairs.
mut. recidiva (selfed)	1929, KULKARNI	DAVIS (BARTLETT)	⊙ 14; 7 pairs	♀ ♂ ⊙ 14. cf table IX. Throws twin hybrids. Hybrid with ⊙ 14 has complex aB and resembles parents, while aa complex is lethal. Hybrid with 7 pairs has complex BB and is gray non-flowering. *Recidiva* is mutant of *pratincola*, table VI.
pratincola E mut. "formosa" × pratincola C	1929, "	DAVIS	⊙ 12 and 1 pair	♀ ⊙ 14. cf table IX. ♂ ⊙ 14. cf table IX.
mut. recidiva × mut. simulans	1929, "	DAVIS (BARTLETT)	⊙ 14; ⊙ 10 and 2 pairs	♀ ⊙ 14, cf table IX. ♂ ⊙ 14; ⊙ 12 and 1 pair. cf tables VI, IX, XI. Hybrid with ⊙ 14 has complex aB (*recidiva* with red buds). The aa complex is lethal. Hybrid with ⊙ 10 and 2 pairs is gray form with complex BB.
mut. simulans × mut. recidiva	1929, "	DAVIS (BARTLETT)	⊙ 10 and 2 pairs; ⊙ 14	♀ ♂ cf above. Hybrid with ⊙ 10 and 2 pairs has complex aa. Hybrid with ⊙ 14 has complex aB. *Simulans* a is said to be different from *recidiva* a.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
eriensis×ammophila	1929, SHEFFIELD	GATES	⊙ 12 and 1 pair	♀ ⊙ 14. cf table IX. ♂ ⊙ 14 less common; ⊙ 12 and 1 pair. Latter is common and "like pollen parent" (GATES). cf tables VI, IX, XI.
ammophila×eriensis	1929, "	"	⊙ 14	♀ ♂ cf. above. Resembles pollen parent (GATES).
ammophila×novae-scotiae	1929, "	"	⊙ 14	♀ cf above. ♂ ⊙ 14; ⊙s 6, 8 cf tables VII, IX, XI.
eriensis×rubricalyx	1929, "	"	⊙ 12 and 1 pair	♀ cf above. ♂ ⊙ 6 and 4 pairs; ⊙ 4 and 5 pairs. cf tables II, III, XI.
rubricalyx×novae-scotiae	1929, "	"	⊙ 12 and 1 pair	♀ ♂ cf above.
franciscana×franciscana sulfurea	1931, EMERSON	DAVIS	⊙ 4 and 5 pairs	♀ ⊙ 4 and 5 pairs; 7 pairs; chain of 4 or more. cf tables I, II, XI. ♂ ⊙ 12 and 1 pair; 7 pairs; cf tables I, VI, XI. One plant of pedigree 58-1 with red buds gave the ⊙ 4 and 5 pairs. Complex *franciscana. s-d-franciscans.*
franciscana sulfurea×franciscana	1931, "	"	7 pairs	♀ ♂ cf above. One plant of pedigree 59-1 with red buds gave 7 pairs. Complex *franciscans.s-d-franciscans.*
(franciscana×franciscana sulfurea) F_2	1931, "	"	⊙ 4 and 5 pairs; 7 pairs	♀ ♂ cf above. In this F_2 of 58-1 two plants showed ⊙ 4 and 5 pairs and one plant showed 7 pairs.

TABLE XII—*Continued*

F_1 HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(franciscana × franciscana sulfurea) F_2	1931, EMERSON	DAVIS	7 pairs; and 1 haploid	♀ ♂ cf above. Of ten plants with red budded cones, one (287-1) of 58-1, was haploid; others (287-3, 5, 7, 10, 31 and 288-1, 4, 81) had each 7 pairs.
(franciscana × franciscana sulfurea) F_2	1931, "	"	⊙ 10 and 2 pairs; 2 pairs, trivalent group and chain of 7 or 8	♀ ♂ cf above. Of four plants with green bud-cones (288-5, 20, 71, 60) of 58-1, three showed ⊙ 10 and 2 pairs. Complex *sulfurea franciscans.s-d-franciscans.*
franciscana sulfurea × latifrons	1928, CLELAND	?	⊙ 6 and 4 pairs	♀ cf above. ♂ 7 pairs. cf table I.

Table XIII consists of twenty-five hybrids grouped separately because they are triple heterozygotes especially prepared genetically by Professor SHULL. They consist of one group of twenty-one *lamarckiana* types and a smaller group of four non-*lamarckiana* types.

Nineteen of the twenty-one *lamarckiana* types have shown one or more configurations also seen in *lamarckiana*. The other two of this group have thus far shown only 4 pairs and ⊙ 6. Two of these twenty-one *lamarckiana* types have shown two types of configurations in the same bud, one case in cells four sections apart on the slide, and another in adjacent cells of the same loculus, respectively. Such a condition would make it difficult to assume that any particular type of closed circle is associated with a particular phenotypical constitution.

Of the non-*lamarckiana* group, all four F_1 offspring show configurations resembling one or both parents. One configuration of this group consists of 7 pairs which indicates that complete pairing is possible in spite of triple heterozygosity.

The data presented in tables XII and XIII offer additional evidence in favor of the hypothesis that chromosomal configurations in the Oenotheras are inherited characteristics due to genic action.

TABLE XIII

CHROMOSOME CONFIGURATIONS OF OENOTHERA TRIPLE HETEROZYGOTES ESPECIALLY MADE UP BY SHULL

LEGEND FOR ABBREVIATIONS:

ang, angustifolia	R, rubricalyx (dominant)
ammop, ammophila	rc, rubricalyx
bull, bullata	sp, supplena
br, brevistylis	sten, stenophylla
ery, erythrina	A, aurata (flower color)
f, franciscana	S, sulfur flower color
fun, funifolia	V, vetaurea ("old gold" flower color)
lam, lamarckiana	Y, yellow flower color
oi, "outside in"	o, long style
p, pervirens	

FORMATION OF HYBRID	STUDIED BY ILLICK IN	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATION	PARENTAL CHROMOSOME CONFIGURATIONS
	Lamarckiana types: Since these parents are all *lamarckiana* types, they might be expected to show one or more of its configurations, 7 pairs (?); 3 pairs and chain; 2 pairs and ⊙ 10; 1 pair and ⊙ 12; chain of 14.			
lam.Y.o.×bull. Y.br.	1930	SHULL	⊙ 12 and 1 pair (from different plants)	♀ ♂ *lamarckiana* types.
lam.Y.o.×Y.br.	1930	"	⊙ 12 and 1 pair	♀ ♂ *lamarckiana* types.
lam.Y.o.×lam.V.oi.	1930	"	⊙ 10 and 2 pairs	♀ ♂ *lamarckiana* types.
lam.Y.×rc.A.oi.	1930	"	⊙ 12 and 1 pair (from different plants)	♀ *lamarckiana* type. ♂ Derivative of *franciscana sulfurea* which shows 7 pairs; ⊙ 12 and 1 pair.
lam.S.o.×Y.oi.	1930	"	⊙ 10 and 2 pairs	♀ ♂ *lamarckiana* types.
lam.A.×lam.Y.oi.	1930	"	⊙ 8 and 3 pairs (from different plants)	♀ ♂ *lamarckiana* types.
p.Y.o.×rc.A.oi.	1930	"	⊙ 6 and 4 pairs (from one plant) or ⊙ 12 and 1 pair; or ⊙ 6 and 4 pairs (from different plants)	♀ *pervirens* (*lamarckiana* type). ♂ cf above.
p.Y.o.×p.V.oi.	1930	"	⊙ 6 and 4 pairs	♀ ♂ *pervirens*.

TABLE XIII—*Continued*

FORMATION OF HYBRID	STUDIED BY ILLICK IN	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATION	PARENTAL CHROMOSOME CONFIGURATIONS
p.V.o.×Y.oi.	1930	SHULL	⊙ 12 and 1 pair, ⊙ 6 and 4 pairs (in one plant), and ⊙ 12 and 1 pair (in one plant)	♀ *pervirens* (*lamarckiana* type). ♂ *lamarckiana* type.
p.V.o.×bull.Y.br.	1929	"	⊙ 12 and 1 pair (from two different plants)	♀ ♂ *pervirens*, selfed.
p.V.o.×fun.Y.br.	1929	"	⊙ 12 and 1 pair, and ⊙ 14 (in same bud and four sections apart on slide)	♀ ♂ *pervirens*, selfed.
p.V.o.×rc.S.V. br.sp.	1929	"	⊙ 12 and 1 pair (in one plant)	♀ ♂ *pervirens*, selfed.
p.sp.V.×bull.Y.br.	1929	"	⊙ 12 and 1 pair (in one plant)	♀ ♂ *pervirens*, selfed.
ang.Y.o.×bull.Y. br.	1930	"	⊙ 12 and 1 pair (in two different plants)	♀ *lamarckiana* type. ♂ *pervirens* (*lamarckiana* type).
ang.Y.o.×V.br.	1930	"	⊙ 12 and 1 pair	♀ ♂ *lamarckiana* types.
ang.Y.o.×rc.A.oi.	1930	"	⊙ 12 and 1 pair (in two different plants)	♀ *lamarckiana* type. ♂ Derivative of *franciscana sulfurea* which shows ⊙ 12 and 1 pair, or 7 pairs.
sten.Y.o.×V.br.	1930	"	⊙ 12 and 1 pair (in different plants)	♀ ♂ *lamarckiana* types.
ammop.Y.o.×rc. A.oi.	1930	"	⊙ 8 and 3 pairs	♀ *lamarckiana* type. ♂ cf above.
ammop.×rc.A.oi.	1930	"	⊙ 6 and 4 pairs	♀ *lamarckiana* type. ♂ cf above.
rc.Y.o.×R.V.br.	1930	"	⊙ 10 and 2 pairs (in two different plants); or ⊙ 12 and 1 pair and ⊙ 14 (in adjacent cells of same loculus)	♀ ♂ *lamarckiana* types.
rc.S.o.×V.br.	1930	"	⊙ 12 and 1 pair (in two different plants)	♀ ♂ *lamarckiana* types.

TABLE XIII—*Continued*

Formation of hybrid	Studied by Illick in	Source of material	Chromosome configuration	Parental chromosome configurations
	Triple heterozygotes of non-*lamarckiana* types			
f.Y.o.×rc.A.oi.	1930	Shull	⊙ 4 and 5 pairs	♀ ⊙ 4 and 5 pairs; 7 pairs; chain of 4 or more. cf tables I, II, XI. ♂ Derivative of *franciscana sulfurea* which shows ⊙ 12 and 1 pair; 7 pairs.
f.S.o.×lam.V.oi.	1930	"	7 pairs	♀ ⊙ 12 and 1 pair; 7 pairs. cf tables I, VI, XI. ♂ ? F_1 is a triple heterozygote with 7 pairs.
ery.Y.o.×rc.V.br.	1930	"	⊙ 6 and 4 pairs (common); ⊙ 8 and 3 pairs	♀ ⊙ 6 and 4 pairs. cf table III. ♂ ?
biennis L.×rc.A.oi.	1930	"	⊙ 10 and 2 pairs and ⊙s 6, 8 (in different plants)	♀ ⊙ 14; ⊙s 6, 8; ⊙ 4 and loose chromosomes; 3 pairs and loose chromosomes. cf tables VII, IX, XI. ♂ cf above.

Discussion

In regard to the *Oenothera* problem the two following facts, from cytological and genetical studies respectively, should be kept in mind: (1) the presence of closed circles which are only relatively constant for any one species; and (2) the genetical phenomena whereby great numbers of old parental types or combinations and relatively few new combinations often appear in the offspring of crosses, indicating some kind of linkage or determinate assortment.

The question arises, do closed circles in the Oenotheras bear any relation to their peculiar genetical behavior? Or in other words, is chromosome cohesion the basis of this linkage or determinate assortment of factors? The writer believes that any attempt to explain these two phenomena should be kept separate since evidence is available which permits such a separation.

DARLINGTON (18) recognizes that "in spite of the hybridity of the ring, pairing is extraordinarily regular. Many homozygous plants which normally form bivalent rings (*Primula*, *Matthiola*, *O. deserens*, HÅKANSSON 38) also produce, by failure of the chiasma in one arm of the paired chromosomes, a rod united by a single chiasma;" nevertheless he holds, as a general rule, that "ring forming segregates of *Oenothera* crosses are hybrids, while bivalent forming segregates are homozygous."

By carefully studying these thirteen tables it is to be noted that complete pairing of the chromosomes apparently takes place equally as well among recognized heterozygous individuals as among those which are said to be homozygous. This would seem to rule out homozygosity and heterozygosity as factors relating to the formation of bivalent chromosome pairs or closed circles.

Table XI lists twenty-nine individuals, each of which has shown *two or more chromosomal configurations*. The complexes for eleven of these are known and are given in each instance, one complex for each individual. In other words, two or more chromosomal configurations have been observed in each of eleven Oenotheras, each of which had only one known complex. In table XII there are four crosses, the F_1 of each showing two or more different types of chromosomal configuration when only one complex is recognized. *Lamarckiana*×*grandiflora* gives an F_1 with ⊙s 10 and 4 (CLELAND and OEHLKERS) and 7 pairs (GERHARD) with the complex *truncans.velans* in each instance. *Lamarckiana*×*grandiflora* also gives an F_1 with ⊙ 14 (CLELAND and OEHLKERS) and ⊙ 10 and 2 pairs (GERHARD) with the complex *acuens.gaudens* in each case. *Grandiflora*×*lamarckiana* gives an F_1 with ⊙s 6, 4 and 2 pairs (GERHARD) and *lamarckiana*×*grandiflora* gives an F_1 with ⊙ 4 and 5 pairs (GERHARD), in both of which there is the complex *acuens.velans*. And *suaveolens*×*strigosa* gives an F_1 with ⊙ 4 and 5 pairs (CLELAND and OEHLKERS) and ⊙ 12 and 1 pair or 7 pairs (OEHLKERS) with the complex *flavens.stringens* in each instance.

Additional data bearing on this question are from *pervirens*, selfed and a *lamarckiana*-type in table XIII. The complex for *pervirens* is doubtless *velans.gaudens;* the complex for the other is not known. *Pervirens*, selfed has shown ⊙ 14, and ⊙ 12 and 1 pair *in cells on the*

same slide four sections apart, and the *lamarckiana*-type individual has shown ⊙ 14 and ⊙ 12 and 1 pair *in adjacent cells of the same loculus*. *Germanica* from table XI should also be mentioned in this connection. Its complex is not available. It has shown ⊙ 14, ⊙ 12 and 1 pair, and ⊙ 10 and 2 pairs, all "in one and the same slide."[2]

This evidence would seem to indicate that the complexes, as such, bear no particular relation to the types of configurations in particular species.

It has been suggested by some investigators that the production of numerous old parental combinations or apparent linkages is due to chromosome cohesion in the form of closed circles, and that the chromosomes are arranged in a determinate fashion. Special studies are now in progress which will definitely decide the question of a determinate or indeterminate arrangement of the chromosomes in closed circles of the Oenotheras. If the arrangement should be found to be indeterminate (SHULL **60**), we can conclude that closed circles are not related to the peculiar genetical behavior of the Oenotheras, and that some other explanation must be found to account for the unusual genetical behavior. Some preliminary evidence has been given (ILLICK **42**) which indicates an indeterminate arrangement of the chromosomes. Such an arrangement would permit segregation in spite of closed circles. The case of *grandiflora* Ait., a "complex heterozygote," has already been mentioned in table IX as one which has thus far shown three types of configurations (⊙ 14, ⊙ 12 and 1 pair, and 7 pairs) and at the same time has shown segregation of two forms (the third being non-viable) in each generation with no apparent regard to the type of chromosomal configuration. HOEPPENER and RENNER (**40**) hold that the principle that chromosome cohesion is at the basis of extensive linkage in *Oenothera* should be viewed with reserve, inasmuch as the flower-size factor behaves independently of the presence or absence of large circles.

The idea of segmental interchange has been presented to explain the peculiar genetical behavior of the Oenotheras. Those who offer

[2] In regard to the three configurations in *germanica*, LELIVELD claims they are the consequence of fixation, but she does not state which should be the normal configuration. I have disregarded this view throughout this study since it does not seem likely that the same fixation should cause such differences among the chromosomes of the same bud.

this hypothesis based upon work with *Datura* and *Drosophila* (BLAKESLEE 1, DARLINGTON 17, 18, and MÜLLER 48) also hold that closed circles result from such interchange between non-homologous chromosomes. SHEFFIELD (55) does not believe that there is any real parallelism between *Oenothera* and *Datura*, and that DARLINGTON'S theory concerning the *Oenothera* situation, based on a comparison with chromosome linkage in *Datura*, which assumes that the ring formation of the chromosomes results from segmental interchange between non-homologous chromosomes, has no advantage.

MÜLLER (48) has presented evidence, based upon studies on *Drosophila* involving the second and third chromosomes, which indicates a determinate rather than a random action on the part of the characters studied, and consequently resulting in fewer new combinations than would be expected on the basis of independent assortment. It is further suggested that the absence of closed circles in *Drosophila* is due to the fact that it is a dioecious individual and therefore less likely to show such a condition than a monoecious form. *Oenothera* is monoecious and therefore, it is claimed, closed circles would be expected more often. But if reference is made to table XIII, there will be found a number of *Oenothera* crosses especially prepared by Professor SHULL. These are *not selfed*, as is true for many monoecious individuals, and thus simulate *Drosophila* crosses. Yet closed circles continue to be found in the F_1 of such crosses. The Copepod (*Diaptomus castor*) is said to have a ♂ 6 and 14 pairs of chromosomes (MATSCHECK 47) and is a dioecious individual. It would appear therefore that the dioecious and monoecious conditions play no particular part in the relative absence or abundance of closed circles of chromosomes.

It should be noted that the data presented in connection with each of these thirteen tables permits the assumption that genic factors are involved, and that chromosome cohesion is a heritable trait and bears no relation to the genetical behavior of the individual in other respects. This suggestion was first made by SHULL and later by ILLICK (41). CLELAND (14) claims that in the study of eighteen true reciprocal hybrids, "in every case the reciprocals have been found to have identical chromosome configurations." This condition might

well be expected on the assumption that chromosomal configurations are heritable traits. SHEFFIELD (**55**) in a recent paper also suggests that chromosome linkage may be inherited as a genetic character, and that an examination of all available data shows nothing contrary to such a possibility.

Segmental interchange eventually may be shown to be the factor involved in the production of large numbers of parental types and the few new combinations in the Oenotheras, as has been found for *Drosophila*, and without assuming it to be a direct causal factor in the formation of closed circles. The relation that segmental interchange would doubtless have in connection with the formation of closed circles according to my hypothesis is an indirect one. The genes that are assumed to be the direct causal factors could, by segmental interchange, be transferred to other chromosomes, thus making possible other types of chromosomal configurations in the succeeding generation.

Summary and conclusions

1. The evidence from tables I to XI indicates that chromosomal configurations are only relatively constant and not necessarily characteristic for any particular species.

2. Three forms, *germanica* (table XI), *pervirens*, selfed (a triple heterozygote, table XIII), and a triple heterozygous *lamarckiana*-type (table XIII) have each been observed to show two or more different configurations. In the first were seen three different configurations in the same slide (⊙ 14, ⊙ 12 and 1 pair, and ⊙ 10 and 2 pairs); in the second, two different configurations were observed in two cells of the same bud four sections apart (⊙ 14 and ⊙ 12 and 1 pair); and in the third form there were seen two different configurations in adjacent cells of the same loculus (⊙ 14 and ⊙ 12 and 1 pair). Twenty-seven other species, mutants, etc., have also shown two or more chromosomal configurations.

3. Whenever variable chromosomal configurations have occurred there have been recorded no particular phenotypical changes with which they might be associated.

4. The presence of seven complete pairs of chromosomes in rec-

ognized heterozygotes indicates that heterozygosity plays no particular part in the formation of closed circles.

5. The evidence seems to indicate that the complexes, as such, as recorded in tables XI, XII, and XIII, bear no special relation to particular chromosomal configurations.

6. It is therefore assumed that the formation of closed circles in the Oenotheras bears no particular relation to their observed genetical behavior. An indeterminate arrangement of the chromosomes in closed circles would offer additional and decisive evidence in support of this assumption.

7. The evidence presented permits the assumption that closed circles are due to genic action as are phenotypical characters and are therefore heritable traits.

8. The assumption that segmental interchange is the factor involved in the production of the unusual number of old parental combinations and the few new combinations is believed to be compatible with the assumption that cohesion of chromosomes to form closed circles is due to genic action.

9. These two phenomena, chromosome cohesion to form closed circles and the unusual genetical behavior in the Oenotheras, are therefore assumed to bear no direct relation to each other.

10. Segmental interchange might possibly have an indirect relation to the formation of closed circles, in that the genes assumed to be the direct causal factors could, by segmental interchange, be transferred to other chromosomes, making possible other types of configurations in the succeeding generation.

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UNIVERSITY OF NANKING
NANKING, CHINA

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ROOT SYSTEM OF QUERCUS MACROCARPA IN RELATION TO THE INVASION OF PRAIRIE

J. E. Weaver and Joseph Kramer

(with ten figures)

Introduction

Invasion of trees into grassland is primarily a phenomenon of plant competition. It involves the relation of the invaded and the invader to light and other atmospheric factors of the habitat, both physical and biotic. But perhaps even to a greater degree the edaphic factors, and especially water in dry climates, are concerned. Consequently to understand the reasons for plant distribution and the changes resulting from natural successions, a knowledge of the parts of the plants underground and of the interrelations of the root systems is of fundamental importance.

The great tension zone or ecotone between the deciduous forest formation of the eastern United States and the grassland formation that centers west of the Missouri River occurs, in part, in eastern Nebraska. It offers a convenient and fascinating field for the study of the age-long struggle between grassland and woodland for possession of the territory bordering the Missouri River. Here the many factors of soil and climate, controlled by limited humidity above ground and scarcity of water beneath, offer conditions under which it is just possible for sturdy pioneer trees to ecize but never to develop to the stature of their kind under more congenial conditions of growth. Against their invasion *Andropogon* and other grasses compete most severely and in the main successfully.

Much work has been done on the root habits of the prairie vegetation; some studies have been made of the underground parts of the bordering chaparral; but little is known of the root relations of deciduous forest trees, except in the seedling stage of growth. To understand the problem of the invasion of trees into grassland, some knowledge of the soil and climate and of the character of the grassland is indispensable.

SOILS AND CLIMATE

The soils of eastern Nebraska, where these studies were made, are rather diverse in character. They include the mellow loess soils immediately bordering the Missouri River, and also those of the drift hills and of the alluvial plains and terraces. Whether the Wabash silt loam of the valleys or the Carrington silt loam of the uplands or other soil types are concerned, they all agree in having great depth and a constantly moist subsoil. After studying the root habits of different types of plants in a wide range of habitats, it has been found that various types of soils may influence the environment in a very similar manner. The ecological significance of various soil types is related to their ability to supply water and the necessary nutrients to the vegetation. The cover of grassland, for example, is very similar in species, structure, and manner of growth throughout much of eastern Nebraska. The various types of prairie that do occur are clearly related to water content of soil, but only obscurely, if at all, to soil type (cf. **18**).

In general, the soils are deep, fertile, fine-textured loams of high water-holding capacity and are circumneutral in reaction. They readily absorb water and the subsoil is always moist to great depths. The mean annual precipitation varies from 28 to 33 inches, nearly 80 per cent of it falling during the growing season. Periods of drought of 15–30 days' duration are likely to occur at any time, and especially after midsummer.

Studies extending over a period of 12 years have been pursued at Lincoln, Nebraska, where the mean annual precipitation is 28 inches (**30**). It has been shown that the available water content in the surface 6 inches of upland soil, which supports a growth of the climax prairie vegetation, was reduced to less than 5 per cent at one or more times nearly every year; but only twice during this period was it reduced to the hygroscopic coefficient. In the second 6-inch level, available water content exceeded 5 per cent three-fourths of the time but was frequently reduced to 2–3 per cent. At no time, however, was the water available for plant growth entirely exhausted. In the second, third, and fourth foot, where water content was less variable, the available supply usually ranged between 5 and 15 per

cent. The maximum was 21 per cent and a few times the minimum fell to 1–3 per cent. In lowland prairie the available water content was 3–10 per cent greater in the surface foot and 5–11 per cent in excess of that of the upland in the deeper soil.

Under an increasing rainfall eastward (maximum 33 inches in Nebraska), the water content has been found to be rather consistently somewhat higher. This is indicated by the greater abundance and wider distribution of the less xeric prairie species, by the growth of chaparral, and by the appearance of the forest. Intermittent determinations of the water content, often during root examinations where excavations 8–22 feet in depth were made, have shown that the soil below 4 feet is also rather moist. It is upon the direct supply of water from rainfall that invading upland forest trees depend. They are not furnished with moisture from the water table (**7**).

Not only is the soil relatively dry when compared with that of the main body of the deciduous forest, but also the air has a great power of desiccation. It is a region with much sunshine, high summer temperatures, high evaporation, much wind movement, and relatively low humidity. During March, April, and May there is approximately 60 per cent sunshine; but June, July, and August have 72 per cent or more. Average day air temperatures sometimes reach 90°F., and maximum air temperatures sometimes reach or exceed 100°F. More usually the average day temperatures are from 75° to 85°. The average night temperatures are 10° or more lower. Evaporation rarely falls below an average weekly loss of 10 cc. per day and is more usually 20–30 cc. During periods of drought, however, it sometimes reaches 40–55 cc. Wind movement is rather constant and often high, and is an important factor in promoting water loss. The average day humidity ranges between 50 and 80 per cent during years of greater rainfall but falls frequently to 40–50 per cent during drier years. The average night humidity is frequently 20 per cent higher. These combined conditions promote a high rate of transpiration and great evaporation from the surface soil. These water losses have been found to average nearly a pound of water per square foot of soil surface per day (**28**). Thus water content in the first 6-inch layer of soil varies widely and rapidly, often 10 per cent or more during a single week.

THE PRAIRIE

The grasslands, before they were broken, formed an almost uninterrupted cover over most of the area. The dominant species of lowlands is the big bluestem (*Andropogon furcatus*). This grass is distinctly a sod former, owing to extensive propagation by rhizomes. It grows throughout the entire warm season and forms dense stands 6 to 10 feet in height. Indian grass (*Andropogon nutans*), tall panic grass (*Panicum virgatum*), and nodding wild rye (*Elymus canadensis*) are the principal grasses accompanying it. They have rather similar habits and stature. Near the forest border, owing to a greater supply of water, this type of prairie extends far up the slopes and often entirely over the hills. The grasses are intermixed with many legumes, composites, and other forbs, which are, however, always of minor importance. Although only 12–20 per cent of the soil surface is actually covered, no large spaces are left unoccupied; and the foliage is so dense that the apparent cover is nearly always 90 to 100 per cent (**27**).

The soil is thoroughly occupied by roots. The really magnificent network of the roots of the grasses completely threads the soil to depths varying from 5 to 10 feet. In the surface especially they form a dense sod. Most of the forbs absorb at even greater depths than the grasses; many extend their root systems to 12–16 feet and some much deeper. The soil is so thoroughly occupied that invasion, even by the roots of trees, seems impossible. The root systems are layered, so that the areas of water control do not too greatly overlap and become too severely competitive.

Smaller areas bordering the forest are held by upland types of grassland. Chief among these is the little bluestem (*Andropogon scoparius*) type. This species ordinarily forms an interrupted sod, the mats or tufts being so dense that few other plants can invade. Accompanying grasses (*A. furcatus*, *Poa pratensis*, etc.) and forbs grow between the mats. Ordinarily only 12–20 per cent of the soil surface is covered by the bases of the plants, but the tops spread so widely that the apparent cover is 80–100 per cent (**31**). Although the plants in this type of prairie are not so tall (0.5–4 feet) nor the vegetation so dense, yet under ground the space is even more thoroughly occupied. The roots of the grasses are finer and have more

and longer branches, undoubtedly an adaptation to secure sufficient water from these drier uplands. The general level of the mat of grass roots scarcely exceeds 3–5 feet, but the deeper soil is well filled with the more extensive roots of numerous accompanying forbs. Many of these are regularly 8–12 feet deep and some 16 to over 22 feet.

It is with such dense, deeply rooted vegetation that an invading species must compete. The invader must not only endure the shade of the grasses, which is often considerable and not infrequently fatal, but also must wrest from the soil the rather scant water supplies in competition with a wonderfully developed system of absorbing organs. Frequently such invasion is accomplished by shrubs that fringe the forest, and the territory thus gained is later appropriated by the trees.

Invasion by chaparral

The more protected ravines of the prairie frequently contain one or more species of shrubs that constitute the marginal chaparral of the deciduous forest. With an increase of soil moisture as the forest margin is approached, these shrubby plants not only become greater in number of species and stature but also they migrate up the slopes and over the hilltops and often form a more or less continuous, narrow border a few rods to a few miles in width between grassland and forest (**3**). Of the numerous species concerned, the three most important are smooth sumac (*Rhus glabra*), coralberry (*Symphoricarpos symphoricarpos*), and hazelnut (*Corylus americana*). Where they are found in common their zonal distribution on slopes shows *Rhus* to be the most xeric. It is also found farthest from the forest margins and is not very tolerant of shade. *Corylus* is the most shade-enduring and does not pioneer far ahead of the forest border. *Symphoricarpos* is somewhat intermediate.

Rhus glabra spreads into grassland by means of very long underground stems, some of which give rise to new shoots 20 to 30 feet from the parent plant. These stems extend outward from the established area usually at depths of only 3–12 inches. The main roots from old plants extend downward and outward under the grass sod, some finally reaching depths of 7–8 feet; but throughout their course they give rise to numerous branches. Many of these, and sometimes the main roots as well, turn obliquely upward and thread the sur-

face soil with a vast network of absorbing laterals (**25**). Thus the roots compete severely with those of the grasses for the water which supplies a growing system of shoots. These shoots ultimately coalesce into a closed canopy above the grasses, which finally disappear as a result of shading.

Symphoricarpos symphoricarpos grows in clumps to a height of only 2 to 5 feet. Its shade is frequently so dense as to exclude even the very tolerant *Poa pratensis*, although this and other grasses may occur between the clumps. The root system is usually only 4–6 feet deep, but lack of linear extent is amply compensated by a well developed absorbing surface. The finest rooted grasses alone excel this species in its thorough occupancy of the soil. The area is extended under ground by efficient rhizomes, and above ground runners play a similar but less effective part. The chief means of invasion are shading and establishment of new plants among the invaders by vegetative propagation.

Corylus americana spreads by means of large, woody, underground parts 4–6 inches below the surface of the soil. These give rise to new shoots 1 to 2 feet beyond the parent plant. An abundance of new roots also arise from the rhizomes. Some of the smaller roots run vertically toward the surface and branch profusely into very fine ultimate laterals, for *Corylus*, like the preceding shrubs, is a strong competitor of the grasses for the water in the surface soil. Competition for water is keen at all levels to which the grasses penetrate, the roots of *Corylus* usually extending deeper than those of the grasses. Above ground the grasses disappear in the shade of the overhanging branches which lean outward 1 to 3 feet. In this marginal territory, the prairie grasses first cease to form seed and then die. Often new shoots of *Corylus* spring from rhizomes that have extended well into the marginal area. The additional shade cast by these increases the handicap.

The intrusion of the shrubs is by mass invasion. As new plants invade by rhizomes, a mulch of leaves is held among the stems. This follows closely upon the periphery of the advancing shrubs and is very effective in further handicapping the growth of the already weakened plants of the prairie. An advance of only 2 or 3 feet may

require 3 to 5 years, so delicate is the balance between invaded and invader (**8**).

The occupation of grassland by forest is often attained only as a result of changes in the habitat brought about by the shrubs. But in many places the bur oak is invading the grassland without the aid of the shrubs. To understand the phenomenon of successful invasion of chaparral or grassland by trees, a knowledge of the root systems of trees is of primary importance.

Root systems of trees

Very little work has been done in America on the root systems of mature trees. In Europe this study has been pursued rather vigorously, especially in Germany, Finland, Russia, and adjacent countries. Most of these studies have centered about coniferous species and much less attention has been given to the trees of deciduous forest. Some of these findings will be discussed later, but a complete bibliography need not be given since such studies have only recently been well summarized. An excellent résumé of the work of European investigators is given by Büsgen and Münch (**6**), and a much more elaborate one by Laitakari (**14**), in his monograph on the root system of *Pinus sylvestris*. He reviews the European investigations from the time of Hartig (1851) and also includes some American work. More recently Stevens (**22**) has given a historical review of both European and American investigations on root growth and root competition of trees.

It is gratifying to note that American foresters are beginning to realize that the major problems of silvicultural practice are intimately related to root growth. Only a beginning has been made, however, and the study of the root systems of trees is at best almost a virgin field in America. The great amount of labor involved in the excavation of even a single mature root system has probably been the principal deterrent factor to such investigations (fig. 1).

Some of the best work in the past ten years, the results of which offer immediate application, has been done with the root systems of fruit trees. In some cases the entire soil mass penetrated by the roots was removed in 50 cm. cubes. For each tree excavated over

60 tons of soil was thus handled. The roots from each cube were weighed and graded. This was supplemented by excavating the larger roots and as much fiber as possible, after careful measurement, and arranging and photographing them in their natural position when all had been removed from the soil (**21**).

FIG. 1.—Portion of root system of *Ulmus americana* along an eroding ravine; such trees occur on upland soil along the Missouri River and do not necessarily depend upon the roots reaching water table for constant supply of moisture.

The growth of roots of seedlings of five species of deciduous trees has been studied in eastern Nebraska on three classes of sites. The form of the root system appeared to be hereditary with the species and was more or less closely correlated with the water content of the soil in the location naturally occupied by that species (**12**).

GURSKY (**10**) has shown that even related species of mature trees may differ in their root habits, not only as to penetration into the soil but also as to root type. For example, *Fraxinus excelsior* has the generalized type of root system while *F. pennsylvanica* differs sharply in lacking the vertical taproots.

Although the root habits of a tree are governed, first of all, by the hereditary growth characters of the species, they are often quite as much the product of environment. Indeed, the impression has been gained by certain investigators that in some cases the root form is an expression of soil environment rather than that of inherent tendencies (**24**).

The senior writer has found that competition of grasses has a pronounced effect, not only upon the growth of the seedlings but also upon the form of the root system. Three-year-old trees of *Gleditsia triacanthos*, *Acer negundo*, *Fraxinus lanceolata*, and *Ulmus americana* were examined. The dwarfed plants that had grown in competition with the grasses had a relatively diminutive taproot system with few long branches. Those about which the grasses were kept closely clipped were more extensive and somewhat symmetrical in shape. Where the sod had been removed to a distance of 6 inches on both sides of the trees along another row, the root system was very asymmetrical and 5–9 feet deep. The widely spreading branches showed a marked preference for the mulched area, the maximum lateral spread (3–7 feet) always being found in it and not in the sod on either side (**8**).

According to Luncz (**16**), experimental evidence shows that variations in the root system of the same kind of tree are often greater in different soils than are those of different kinds of trees in the same type of soil. He concludes that the tree itself is not in the main responsible for the development of its root habit, but that this is determined by the composition of the soil and subsoil, nutrients, air, water, competition between root systems, etc. It must accommodate itself to conditions occurring during its life.

Other investigations might be cited, but enough has been given to indicate the need of knowledge of the soil and of the general competitive conditions under which the bur oaks to be described have developed.

Investigation

The oak forest and the soil profile

The Carrington silt loam from which the root systems were excavated is a typical prairie soil. It has been occupied by only one or possibly two generations of trees. These have migrated on to the

uplands from the valleys along streams where they were protected from prairie fires. Most of this migration and invasion of prairie has occurred in the 70 years since the early settlement of eastern Nebraska and the cessation of prairie fires. Three lines of evidence substantiate this fact. Pioneers are still living who well remember that the trees existed only as marginal forests in sheltered places, largely along streams. There are no very old trees found standing today nor mounds or pits to mark their former abode. Finally, the soil is scarcely modified, except in its very surface, from that of the profile of typical prairie soil.

The bur oak forest where the excavations were made is typical of those of the forest margin in eastern Nebraska (fig. 2). It consisted of a pure open stand. The trees were mostly about 50 to 65 years old, 30–40 feet tall and 12–18 inches in diameter near the base. They were rather widely spaced (10–40 feet). The individuals had well developed crowns with large branches and stout, somewhat sparse branchlets. About half of the ground was carpeted with *Poa pratensis*, the shade and leaf litter elsewhere being too deep for this grass. *Corylus americana*, *Symphoricarpos symphoricarpos*, *Rhus glabra*, and *Cornus asperifolia* were the characteristic shrubs, although *Rhamnus lanceolata*, *Sambucus canadensis*, and *Cornus stolonifera* also occurred. Owing to utilization of the forest as pasture land, the shrubby growth was only poorly represented.

The mature soil profile is well developed, showing that it has been undisturbed by excessive erosion, except in very recent years. The soil is of glacial origin. There was only a small percentage (about 10) of particles coarser than fine sand, and the fine sand rarely exceeded 15 per cent. The bulk of the soil at all depths to 12 feet consisted of about equal proportions of very fine sand, silt, and clay, with the clay fraction preponderating at depths beyond 12 inches. Horizon *A*, or zone of extraction, from which the carbonates have been leached and much of the clay alluviated, extends to a depth of about 18–20 inches. It has an excellent granular structure and a water-retaining capacity (HILGARD method) of approximately 60 per cent. A thin layer of humus occurred only in the surface inch. Horizon *B*, or zone of concentration, where much clay has accumulated, is about 22 inches thick, reaching a depth of 40 inches. Much of the lime has been leached from this horizon. The shrinking of

the soil in drying and its cleavage into long perpendicular columns give the soil of this horizon a distinctly columnar structure. At greater depths, in horizon *C*, where neither extraction nor accumulation of materials has occurred as a result of soil-forming processes, the soil has a massive structure. This horizon extends far beyond the depths of the roots of the trees. The carbonate content is high,

FIG. 2.—Portions of main lateral roots of bur oak 40–55 feet in length. The stand of oaks in background is somewhat denser than where excavations were made.

streaks of chalky material being somewhat in evidence. The soil is friable, more easily penetrated by roots than is the columnar layer above, and prismatic cleavage becomes imperfectly developed. With increasing depth the color likewise changes. The very dark brown of horizon *A* intergrades into the grayish brown of the deeper one, and this in turn shows a transition to buff in the massive layer.

ROOT SYSTEM OF SEEDLINGS

In regard to the establishment of the individual tree, it has been shown (**23**) that the initial root habit is an important and often a

determining factor in the survival or death of the seedling. The amount of water and nutrients that will be made available to the seedling depends upon the initial root habit, which it seems is in turn dependent upon the species and is not in most cases at once modified by external conditions.

An adaptation of the bur oak to dry sites is shown in its extending the taproot deeply, usually about 9 inches, before unfolding the leaves above ground (12). Moreover, the root continues to penetrate the soil at a rapid rate and thus avoids succumbing to drought during summer when the surface layers may become dry. The senior writer has traced the development of the seedlings where the acorns were planted among the prairie grasses. A depth of more than 3 feet was attained the first year. More recently HOLCH (12) has studied the root activities of this species in cultivated soil on a cleared hilltop in eastern Nebraska. At the end of the first growing season a depth of 5 feet and a total lateral spread of 30 inches were attained. By the second autumn it had extended 2 feet deeper and the lateral spread had reached 38 inches. An examination of 3-year-old seedlings showed that the taproot had reached a depth of 8.5 feet and some of the main laterals had grown so vigorously that the total spread of branches was 5.2 feet. This rapid growth explains the ability of the bur oak to thrive on upper slopes and exposed hilltops in soil so dry that all other native trees fail. A study of its very abundant laterals which clothe the taproot from near the soil surface to the maximum depth of penetration also explains how this species can compete successfully for water with the shrubs and grasses.

The rapid growth of lateral branches, some near the soil surface, during the third year is of interest. In its younger stages this tree has, generally speaking, only feeding roots; later it develops anchorage roots.

EXCAVATION OF MATURE ROOT SYSTEM

A mature tree 65 years old, 37.5 feet tall and 14 inches in basal diameter, was selected for the initial excavation. A portion of the root system near the trunk was exposed as a result of erosion. This had occurred almost entirely within recent years and after the tree

was nearly mature. It was the result of close pasturing with both cattle and swine.

The general plan of procedure was first to excavate the taproot and those parts of the laterals that occurred within a radius of 12 feet from it. Afterward the branches extending beyond this area were traced. This method was followed in order to reduce, so far as possible, the great labor involved in removing a large volume of soil, the original excavation being filled in the process of obtaining the long lateral branches.

PRIMARY LATERALS.—All of the soil of horizon *A* was carefully removed from the circular area and most of the major roots exposed to a depth of about 2.5 feet (figs. 3, 4). Each primary lateral was numbered and measured, and the number, diameter, and direction of growth of its branches were recorded. The branches were then severed from the primary lateral and temporarily left in place. The main root was cut where it branched from the taproot and also at the place where it left the excavated area, or entered the deeper soil within it. Each root was marked by means of a numbered stake driven into the soil, so that it could be further examined after the taproot and its branches within the primary area of excavation had been studied.

There were 15 major branches, 2–7 inches in diameter, that had their origin on the first 15 inches of the taproot. Seven others, varying from 2 to nearly 4 inches in thickness, arose from the taproot between 15 and 36 inches. The depth of origin, diameter, length within the primary excavation, and diameter at the distal, severed ends are shown in table I.

Table I indicates the large size and widely spreading habit of the main roots. Root no. 12, for example, was 4.5 inches thick. It originated 8 inches below the soil surface, and ran outward a distance of 13 feet where it was still 1.2 inches thick but only 30 inches deep. All but four of the fifteen uppermost roots grew outward at so small an angle from the horizontal that they left the 12-foot excavation on all sides of the tree at an actual soil depth of only 15 to 32 inches. The other four extended more obliquely downward. Among those originating deeper, five of the seven behaved like the first eleven. It must be recalled, however, that the original depth was somewhat

greater, perhaps 16 inches, since the soil on this hillside had been eroded about this particular tree.

The root systems of two other trees, one 18 and one 14 inches in diameter, were excavated to determine whether or not the one described was unusually well furnished with large laterals and also to secure further information on their depth of origin, direction, and depth of growth. The data resulting from this study of the branches originating from the first 40 inches of the taproots are given in table II (cf. fig. 5).

FIGS. 3, 4.—Fig. 3 (above), root system of bur oak as it appeared after removing about 2.5 feet of soil. Fig. 4 (below), same as figure 3 but from opposite side of tree.

An examination of table II shows that the three trees had 22, 27, and 26 main roots respectively over 2 inches in diameter; nine, seven, and seven 1 inch thick; and that the third tree was especially well supplied with branches of smaller size. The depth of origin of the large laterals was also very similar to that of the tree previously described, although only a little erosion had occurred. The bulk of the major laterals in all cases originated in the first 2 feet of soil;

TABLE I

DATA ON MAIN LATERALS TWO OR MORE INCHES IN DIAMETER

ROOT NUMBER	DEPTH OF ORIGIN ON TAPROOT (INCHES)	DIAMETER (INCHES)	LENGTH UNCOVERED (FEET)	DEPTH WHERE CUT (INCHES)	DIAMETER WHERE CUT (INCHES)
1	15	3.8	12.0	20	0.5
2	8	4.2	12.0	15	1.2
3	5	4.1	5.5	20	2.0
4	15	6.2	6.0	26	2.0
5	11	3.9	9.0	26	1.5
6	5	4.0	11.0	24	1.0
7	10	4.8	14.0	30	1.0
8	10	4.7	11.0	32	2.0
9	14	6.2	10.0	31	1.5
10	14	2.0	6.5	30	0.7
11	8	6.9	15.0	29	1.7
12	8	4.5	13.0	30	1.2
13	9	4.0	3.4	20	2.5
14	14	2.7	12.0	29	1.5
15	15	4.0	12.0	32	1.2
16	32	2.0	12.5	24	0.5
17	36	2.5	12.0	30	0.2
18	22	2.2	10.0	32	0.9
19	26	2.5	11.0	38	0.7
20	24	3.0	9.0	37	1.0
21	28	2.0	5.0	33	1.1
22	25	3.7	10.0	31	0.7

many occurred near the soil surface; and relatively few were found at depths greater than 2 feet. The characteristic direction of growth was also similar to that already described; most of the roots ran far outward, some horizontally, mostly with only a slightly downward course.

The major laterals nearly always pursued a direct course, often with long graceful curves, and with few exceptions no abrupt turns were encountered. Moreover the roots tapered gradually and, except where major branches arose, very uniformly. For example, root no. 2, with an original diameter of 4.2 inches, tapered to 3.5

inches at 6 feet, 2.5 inches at 9 feet, and was 1.2 inches thick where cut at 12 feet from its origin. No. 12 was 4.5 inches thick but had diameters of 4, 3.5, and 1.2 inches at 6, 8, and 13 feet respectively.

TABLE II

NUMBER OF MAIN LATERALS OF VARIOUS SIZES (IN INCHES) ORIGINATING ON THE FIRST 40 INCHES OF THE TAPROOTS

TREE	INCHES												
	DIAMETER	0.25 OR LESS	0.25–0.5	0.5–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
1	14	7	3	0	9	7	4	8	0	3	0	0	0
2	18	0	0	2	7	8	7	2	4	2	2	1	1
3*	14	4	5	16	7	8	8	9	0	0	1	0	0

* On this tree 25 of the laterals, nearly all large ones, originated from the first 22 inches of taproot.

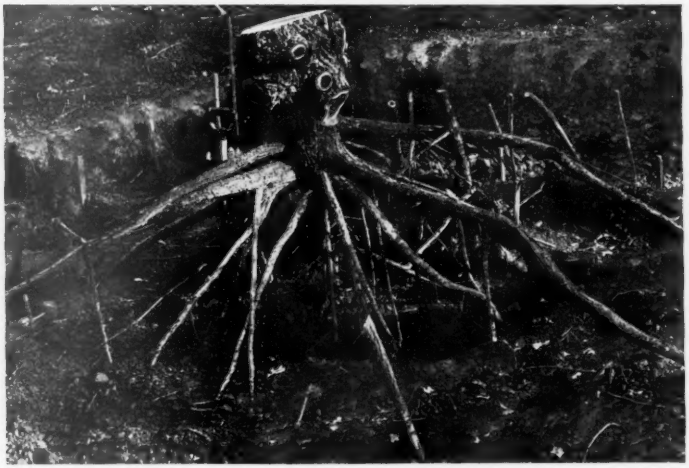

FIG. 5.—Taproot system after the large, upper lateral branches had been removed; note that the branches originating deeper take a more oblique course. Vertical roots are branches (sinkers) from the main laterals. The stakes mark places where the main branches were cut.

Grafting of roots of the primary laterals, and in fact, of roots of smaller sizes, was very much in evidence on all of the trees examined.

SECONDARY LATERALS.—The number of branches of the second order that arose from the parts of the roots thus far unearthed was

great. Their distribution was irregular but most of the larger ones had their origin within 6 to 8 feet of the taproot. The numerous smaller ones in general arose from the more distal portions of the main roots. A total of 82 larger branches ranging in size from 0.25 to 4 inches were counted and measured. Twenty-nine belonged to the first group (0.25 inch), twenty-four were 0.5 inch thick; and the numbers of larger sizes, increasing in diameter by 0.5 inch respectively, were 9, 7, 3, 5, 2, 2, and 1. The direction pursued by these branches was variable. A few ran horizontally; many spread laterally, forming a 20°–30° angle with the main root and then almost paralleled its course. Others ran obliquely outward and downward. These branched similarly to the main roots, which will later be more completely described. But fully one-fifth of the branches grew so nearly vertically downward that they may be characterized as "sinkers."

The branching of the sinkers, which varied in diameter from 0.2 inch or less to 2.5 inches, was characteristic and their downward extent rather great. They were clothed with widely spreading branches, both large and small, which rebranched repeatedly, resulting in a large absorbing area. They may best be understood by a description of a few typical examples (fig. 6).

One sinker originated from a main root 18 inches from the taproot and was one of five similar branches arising at various places in the first 4 feet of its course. Its original diameter was 2 inches. It penetrated almost vertically downward, tapering gradually and rather uniformly to a depth of 7.5 feet where it was only 5 mm. thick. Thereafter the diameter was almost uniform to a depth of 15 feet. A large branch, 0.75 inch in diameter, arose at 12 inches from the proximal end. It ran obliquely downward at an angle of 45°. This branch gave rise to many laterals, for example five 0.25 inch in diameter and twelve 1 mm. thick, in the first 3 feet of its course. A lateral 5 mm. in diameter arose 6 inches above the large one described, and another 0.7 inch thick arose at about the same level. There was a total of thirty branches in the next 5 feet. They arose at irregular intervals, and varied from 1 to 10 mm. in diameter. Nearly all ran outward at angles of 15° to 45° from the horizontal but a few penetrated more vertically downward. They spread widely and branched

profusely. Two laterals, 3 and 5 mm. thick respectively, arose at 7.5 feet depth. The larger one ran obliquely for a short distance and then turned downward and more or less paralleled the course of the main root, the size of which it approximated. Below 7.5 feet the branches were small and rather numerous but short. Only a few threadlike laterals occurred below 10 feet, and just beyond a depth of 15 feet the root ended abruptly in the hard, dry soil.

FIG. 6.—Sinker about 15 feet long in three vertical sections; note rather numerous branches. Running horizontally across the bottom is a portion of one of the numerous ropelike roots.

Another sinker, originating from a major lateral 4 feet from the taproot, was 2 inches in diameter. Like the preceding, it penetrated vertically downward and tapered gradually. Branches of various sizes arose in profusion. Fourteen major branches, 5 to 15 mm. in diameter, and an equal number of smaller ones occurred in the first 4 feet of its course. These had threadlike sublaterals in abundance, all of which were clothed with masses of absorbing rootlets. The direction of growth was similar to that already described. At greater depths the branches became more threadlike and fewer in the harder soil. Maximum penetration was about 15 feet.

Still another sinker gave rise to seven major branches (0.25–0.5 inch thick) and eight smaller ones in the first foot of its course, and

thirty-five in the next 4 feet. It then penetrated to the 10-foot level, branching more sparingly. Since several of the larger branches ran obliquely outward for distances of 10 to 15 feet and all branched freely, the total soil mass penetrated by a single sinker is very great.

While a few of the sinkers examined had no large laterals and relatively few smaller ones, the preceding descriptions are typical of the group. The individual sinker develops in a manner very similar to that of a sapling oak, and if its origin were unknown it might readily be confused with a small taproot system.

TAPROOT.—The taproot was 14 inches in diameter at its origin, that is, near the old soil line and where the large lateral roots originated. But it tapered so rapidly, after giving rise to the numerous large branches, that at a depth of 3.3 feet it was only 4.5 inches thick. Its width decreased to 3.7 inches at 5 feet in depth and to 1.75 inches at the 6-foot level, after giving off a branch equaling its own diameter (fig. 7). In addition to the twenty-two roots in table I, sixteen others occurred on the first 3 feet of the taproot. Ten of these were of large diameter (0.25–1.6 inches); the others were only 2–3 mm. thick. Nearly all penetrated much more obliquely downward than the roots already described. Between the depths of 3 and 7 feet, laterals were much fewer and of smaller size; in fact there were only two with a diameter greater than 1.5 inches. Seven branches were 1–1.5 inches thick; fourteen others were 0.2–1 inch in diameter; and three were of smaller size. This made a total of sixty-four primary branches from the first 7 feet of taproot, of which 60 per cent (including nearly all the larger ones) originated in the first 3 feet.

From the 6-foot level the taproot (now only 1.75 inches thick) branched sparingly and pursued a tortuous downward course. Three branches (included above) arose in the seventh foot. These were 0.75, 0.5, and 0.3 inch thick. Six other small laterals (4–5 mm. thick) were also found. The taproot was now reduced to a diameter of only 0.75 inch. In the eighth foot a single horizontal lateral 0.3 inch thick arose. At 9 feet in depth the main root gave rise to a small branch 4 mm. in width which ran outward and upward. The taproot, of similar small dimension, pursued a tortuous downward course through the hard soil. Another branching reduced its size to 2.5 mm.

and still another to 1 mm. It was now at about the 10-foot level. Only a few other threadlike branches were found at this depth. The brown, brittle, threadlike taproot and several of its branches pur-

FIG. 7.—Portions of two taproots: the one on right is from tree no. 1 and is about 6 feet long; the other, cut at depth of the chalk line on the first, is from tree no. 3 which maintained a larger diameter (note crooked shape).

sued parallel downward courses, branching sparingly. Many of the branches were decayed near their ends and none, including the taproot, penetrated beyond 14 feet in depth.

The rapid tapering of the taproot and its lack of greater penetration initiated further study. Oaks no. 2 and no. 3 were felled and

their taproots examined. That of no. 2 tapered from a diameter of 18 to 5.5 inches in the first 3.3 feet of its course. A foot deeper it was only 1.5 inches thick and at the 5.5-foot level, 0.5 inch. Thus the taproot was again poorly developed.

FIG. 8.—Root system of tree no. 3; taproot shown to depth of about 6 feet

On the third oak the main root lost half of its original thickness (14 inches) in the first 2 feet. At a depth of 5 feet it was only 2.7 inches thick, but at the end of the twelfth foot, the root having been much flattened and distorted for several feet, the diameters were 1 and 2 inches respectively (fig. 8). Here it ran horizontally for some distance, gradually narrowing and finding great difficulty in penetrating the hard soil. It probably penetrated somewhat deeper than

the first taproot examined. It should be noted also that the portion of this taproot between the 3.3- and 12-foot levels was much more poorly branched than the first one described. In fact, only thirteen branches were found on this part. All but four were less than 0.5 inch in diameter.

Several other trees, where the taproots had been partly exposed by erosion, yielded similar data. A much larger specimen with a diameter of 3.3 feet was studied on a high, level terrace which the Weeping Water River had eroded. The taproot system was fully exposed for a depth of 9 feet. It was much smaller than a score of the main lateral roots and measured only 3.5 inches in diameter at 4.5 feet depth. The diameter, however, was nearly as great at the 9-foot level.

From these studies it may be concluded that the taproot of the bur oak, at least in the type of soil where it was studied, is less extensive than many of its major branches.

ROOT DISTRIBUTION NEAR THE TREE; ROOT TYPES.—A clear description of the network of roots occupying the soil within the 12-foot radius of the tree is somewhat difficult. In addition to sixty-four main branches of the taproot, which occurred primarily in the first 2 to 4 feet of soil, there were eighty-two large secondary roots (0.25–4 inches in diameter). About one-fifth of these were sinkers, each often having a root system like that of a sapling oak. In addition, many roots originated from the more vertically descending main laterals which were not uncovered (and hence not included) in the earlier excavation. These, with branches, both large and small, from their secondary laterals, and also the very numerous branches of secondary roots that were originally uncovered, formed an intricate root pattern.

The area within a radius of 10 feet of the tree (except close to the taproot) was especially well occupied by roots of various sizes. Aside from the large, thick roots already described, three other types predominated, and indeed intermediate sizes were relatively scarce. First there were the large ropelike roots, usually 0.5 inch or somewhat less in diameter, which ran long distances (5–20 or more feet) with little decrease in thickness (fig. 6). They and the larger branches, including the main laterals, gave rise to an abundance of

tough, reddish brown, cordlike roots that were usually 3–5 mm. thick, and maintained a uniform diameter for distances of 5 to more than 10 feet. These ran in all directions, often even vertically upward or downward, and threaded the soil with an efficient network which, in part, was the source of the third general type, that is, the capillary branches. While these cordlike roots sometimes ran 2 feet or more with scarcely a single lateral, more usually there were five to eight branches per foot and sometimes as many as twenty to twenty-four.

These cordlike roots (and even finer branches) arose from the larger roots and, in fact, also from the main laterals. Sometimes they were sparse but often abundant. Frequently as many as twelve to twenty per foot were counted; sometimes there were several feet of main roots with none.

In general the first 6 feet of soil was filled with a well developed root system, approaching in places such a network as is often found among herbaceous plants (**26, 29**). The root network decreased rapidly at depths of 6 to 10 feet, and at greater depths roots were sparse.

The smaller laterals, which undoubtedly carried on the major part of the absorption, warrant careful consideration. In fact, the description thus far has dealt with the skeletal framework from which the absorbing roots arose. This is true even where the non-absorbing roots form a tangle below ground (as was found within 10 feet of the tree) similar to a thicket of branched shrubbery and small trees above the soil. Figure 9 shows a few smaller roots and the abundance of much branched rootlets.

Many of these fine rootlets, especially in the surface soil, come from obliquely or vertically ascending branches which often originate several feet in depth. For example, one root 0.4 inch in diameter was cut from a main horizontal lateral. It ran vertically upward 28 inches, gave rise to several large branches, and then turned nearly horizontally in such a manner that many of the ends of the profuse ultimate laterals came within 0.25 inch of the soil surface. Eleven branches 1 to 4 mm. in diameter had their origin in the surface foot of soil. They spread widely, branched repeatedly, and their mats of ultimate rootlets almost filled the rich surface substratum.

Many branches with absorbing rootlets were floated out in water and examined in detail. The following examples are representative of well branched roots. A section of a root 9 inches long gave rise to eight branches 1 mm. thick and forty-two of smaller size. The larger

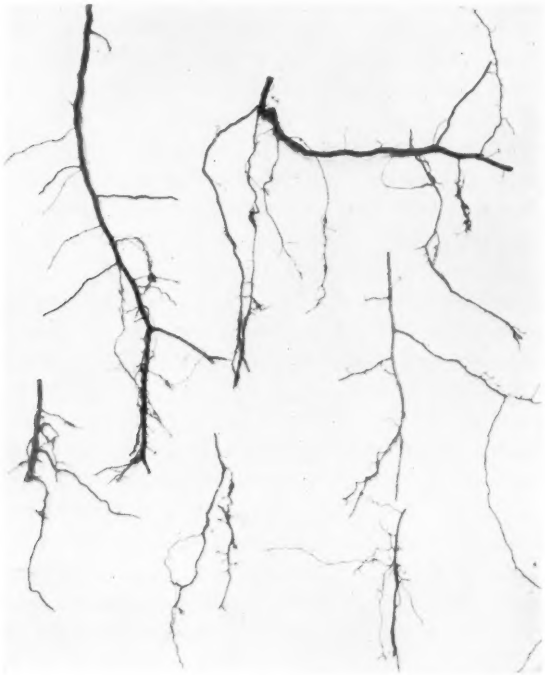

FIG. 9.—Selected fragments of roots showing usual degree of branching

ones rebranched at the rate of twelve rootlets per inch; the finer ones had seventeen to twenty-five fine laterals per unit length. Many of these were rebranched three or four times. Another root section 5 inches long had seven millimeter branches and twenty-eight threadlike ones. Branching at the rate of fourteen laterals per inch was common on fine rootlets, and five or six per inch occurred on those that were only poorly furnished with laterals.

The root termini commonly had as many as twenty branches per inch, mostly 0.5 inch or less in length but so completely rebranched that the soil was extremely well filled. Mycorrhizal mats, yellow, white, or pink in color, were found in great abundance. Here the root mass was often flattened into one plane, forming densely appressed mats or "fans" 0.5–1 inch wide. Often fifty or more short branches per inch occurred in such areas (fig. 10). The mats were especially well developed throughout the first foot of soil but also occurred deeper. The whole horizon *A* within a radius of 12 feet from the tree was well threaded with the absorbing rootlets. As the roots extended many feet farther, repeated divergence of branches continued to afford a skeletal network for the origin of more fine laterals; but here, of course, the whole territory was not so fully occupied by the roots of an individual tree.

FIG. 10.—Ultimate absorbing laterals showing also some mycorrhizal "fans."

As regards color, all of the roots whether large or small were some shade of brown, varying from grayish to reddish brown. They possessed great tensile strength, being characteristically as "tough as oak."

Upon the death and decay of the roots, channels are left in the soil. One channel 0.5 inch in diameter was traced at the 10-foot level for a distance of more than 4 feet. Many smaller ones were found. Some were partially lined with fragments of old bark; others contained a new growth of roots. Undoubtedly the larger channels, at least, remain in place for long periods of time. Dead root ends are often found on vigorous forest trees, and sometimes entire roots or at least considerable portions of them may die and decay.

EXTENT AND BRANCHING HABIT OF PRIMARY LATERALS.—As regards root extent beyond the primary excavation, the following examples are illustrative. Root no. 8 was cut 11 feet from its origin where the diameter was 2 inches. Within a foot it forked dichotomously. One branch ran obliquely downward; the other ran outward almost horizontally and at a depth of about 2 feet. The latter immediately gave rise to a branch that also ran horizontally, making an angle of 25° with the main root. The main root tapered to a diameter of 1 inch 3.5 feet farther out, and after giving rise to two branches each 0.25 inch thick it was only 0.5 inch in width. One of these branches ran vertically downward; the other horizontally outward. Throughout its entire course this root gave rise to many small branch roots 3 to 5 mm. thick. These extended in all directions, as frequently upward as laterally or downward. At a distance of 30 feet from the taproot, and where the main root was only 4 mm. thick, it turned obliquely downward. The main root now divided into numerous smaller rootlets which extended outward and downward approximately 8 feet farther.

The other main branch of no. 8 soon pursued a course more or less parallel with the surface soil at a depth between 2 and 3 feet. It also extended outward (31 feet) before running obliquely downward. Its branching was similar to that of the other fork. The tip was only 3 mm. in diameter and was not traced beyond 5 feet in depth.

A branch of no. 8, 1 inch thick, which arose at a distance of 10.8 feet from the trunk, also ran far outward. It soon branched dichotomously, one branch running downward at an angle of 20° from the horizontal. The other branch was traced outward in the second and third foot of soil 23 feet farther. Here it divided into several branches only 3–5 mm. thick. These branched again and again, running outward and downward about 10 feet more. The root was ropelike and tapered so slowly that for many feet change in diameter was scarcely perceptible. Few large branches were found and smaller ones originated only intermittently. Only six laterals of various sizes arose in the first 5 feet of its course, but on other parts there were twelve to eighteen small branches per foot.

This main lateral with its widely spreading major and minor

branches, all covered more or less profusely with absorbing rootlets, well illustrates a small part of this really wonderful anchoring and absorbing system. Sometimes a root turned upward a foot or more, or abruptly downward, but the curves were nearly always remarkably gradual and graceful compared with those of many other species of plants. The direction of growth of even isolated roots could easily be determined by their branching habit. Roots of all sizes, including the taproots and main laterals, showed a decided tendency to give rise to one or more branches where they did bend abruptly.

Often the main roots enlarged 0.5 inch or more in diameter if the soil was eroded from them or if in their course they appeared at the surface. This was due, in part, to the increase in the development of the thickness of the bark.

Further description of the lateral roots seems unnecessary; in fact, all of them were not examined. Instead, the roots of certain other oaks on gently sloping ground were studied since they were somewhat easier of access. Only two will be described.

The first was 3.6 inches thick at its origin which was 12 inches deep. It ran outward at a rather uniform depth between 2 and 2.5 feet. Thirty feet outward it was still 1 inch thick. It was traced 10 feet farther where it was 5 feet below the soil surface. Here it turned abruptly downward, the diameter increasing to 1.5 inches. It tapered rapidly in the next 3 feet of soil but was not traced to its end. Five large branches (0.25–0.75 inch thick) and very numerous smaller ones arose beyond the 12-foot radius and two between 30 and 40 feet. In addition, smaller laterals were abundant.

A main lateral was examined on another tree that had a trunk diameter of 14 inches. It was 3 inches thick and originated at a depth of 2 feet. This root pursued a gently curving, nearly horizontal course at a depth of about 28 inches. The diameter decreased at 10-foot intervals to 2.75, 2.5, 1.75, 1.25, and 1.0 inches respectively. At 54 feet from the tree it turned directly downward, and although this part was unearthed only a few feet, it probably penetrated deeply. Three major branches 0.5 to more than 1 inch in diameter arose in each of the three distal 10-foot sections. They ran either horizontally or obliquely upward or downward and rebranched in a manner not unlike that of the main root. Almost countless numbers

of small rootlets originate from such a major root and its network of laterals, and the total volume of soil laid tribute by a single major branch is really enormous.

Other main branches were traced for distances of 40 to 56 feet and one to more than 60 feet. This was two to four times greater than the radius of the crown of the tree (fig. 2).

Relative weight and volume of tops and roots

The tree was cut at the original ground line and the top divided into convenient sections for weighing. Although the leaves were drying, few had fallen and their weight was included with that of the trunk and branches. The total weight of the top was 1285 pounds.

The large main roots from the upper portion of the taproot, except a few of the ends, were collected and weighed. The weight of the termini (which was not great) was approximated from the weight of similar roots of average length and equal diameters. All of the major roots, over 5 mm. in diameter, from one-fourth of the area penetrated by the root system below 3 feet, were also collected and weighed and the weight quadrupled. These weights, with that of the taproot itself, gave a total which was just equal to the weight of the tops. Since the soil was dry, the wood of the roots probably contained little more water than the branches. Thus this specimen had a skeletal root system which equaled in weight that of the parts above ground. The significance of this from the standpoint of absorption is not great. It merely indicates the enormous development of the anchorage system and at the same time the extent of the framework upon which the absorbing, fibrous network of roots develops. Had all of these fine roots been collected as well, their weight would have added little to the total, but would probably have caused the weight of the roots to exceed that of the tops.

The relative volumes of roots and tops were also approximated by determining first the weight and then the volume of average samples of roots as well as tops, each of three different sizes. Since the weights of roots and tops were approximately equal, the volume of the roots, because of their greater density, was about one-tenth less than that of the wood above ground.

Discussion

One of the most striking features of the root system of the bur oak is its wide lateral spread. BÜSGEN and MÜNCH (**6**) state: "At the margins of woods, tree roots are not seldom to be met with at a distance of 20 meters in the adjoining fields. For this reason cultivated ground near woods must be protected from the roots of trees by means of trenches."

PEREN (**20**) studied the roots of fruit trees in relation to spacing in the orchard as well as the length of time "filler trees" or bush fruits should be allowed to remain without seriously competing with the permanent trees. A 15-year-old Norwegian cherry had a lateral spread of roots of 30 feet. The roots of certain varieties of 16-year-old apple trees extended to a maximum distance of 24.3 feet from the base of the tree.

GEMMER (**9**) has shown that a long-leaved pine, *Pinus palustris*, 3 inches in diameter and 20 feet tall, had extended its roots 8 feet laterally through the sandy soil. He states that on mature trees roots 0.5 inch in diameter are not infrequently found 30 feet from the tree, a distance twice the radius of the crown.

LAITAKARI (**14**) found that the longest roots of *Pinus sylvestris* occur on sandy heaths. The greatest lengths determined were 17–25.5 meters. These findings are similar to those of ALLAN (**4**), who states that the roots of a matai (*Podocarpus spicata*) 15 meters tall, in a rain forest of New Zealand, had a lateral spread of 19.5 meters from the base of the tree.

There is considerable evidence that the roots of trees developing in poorer, lighter, well aerated soils tend to grow longer and often thinner and straighter than those in more compact and richer soils which are shorter and often thicker, pursuing a more devious course. Since the bur oak grew in fertile, compact, but well aerated silt loam soil, its great lateral extent cannot be attributed to these factors of the substratum. It would seem that the scarcity of the water supply, at least during recurrent periods of drought, is the chief external factor promoting extensive root development. This has repeatedly been determined experimentally for various herbaceous plants, and also undoubtedly holds for trees (**13**).

LAITAKARI (14) finds that the size of the tree and the length of the root system are rather closely dependent upon each other. It thus seems clear that the small size of the trees of these marginal oak forests is limited by the ability of the root system to furnish sufficient water for greater growth. An open stand where the crowns do not touch is indeed a closed one so far as the overlapping root systems and the supply of the dominant limiting factor, water, are concerned.

The more or less vertical growth of roots from the deeper, large horizontal or oblique branches is a rather common phenomenon among trees. For example, it has been found in Europe (HILF 11) that the roots of the beech do not extend beyond the crown but run downward more or less diagonally to various depths. Branches from these deep roots extend upward to the humus layer, where they rebranch profusely in the richer surface layers of soil. In fact, HILF states that notwithstanding the deep skeletal root system, the beech is especially dependent upon the humus layer for its nutrients. PELHAM (19) found that the large primary laterals of the pecan extended widely at depths between 2 and 5 feet. They gave rise to an abundance of vertical "feeding roots" 0.25 inch or less in thickness which extended nearly to the surface of the soil.

The intergrowth of roots is a common phenomenon in many forest trees. It is found most abundantly within a few feet from the base of the tree. Both horizontal and vertical roots are concerned; in fact the two types were frequently grafted one into the other. LAITAKARI (14) states that in the Scotch pine, roots are frequently found which only connect two other roots and do not grow any farther. He suggests that the main purpose of this kind of "bridge-root" seems to be to strengthen the root system. None of these were seen in the oak.

The phenomenon of root intergrowth is easily understood when the very firm structure of the soil is considered. The soils of horizons *B* and *C* were both very hard and difficult to remove, even with a hand-pick. The extreme compactness of the soil about the roots, increasing with root diameter, although not surprising is remarkable. As a result of growth, the taproot and its large branches compressed the soil about them so greatly that it was removed only with much

difficulty. Two young roots coming into contact would exert in their enlargement an enormous pressure, not only against the soil but also against each other. Under such conditions grafting would be almost an inevitable result.

One very noticeable phenomenon observed in the excavation was the small effect a first or second generation of trees had upon the prairie soil. In the columnar layer there was, as in the case of herbaceous plants of the prairie, a marked tendency of the roots to follow the cleavage planes between the columns and form a network of absorbing rootlets which spread out over their surfaces. BURGER (**5**) has shown the important part played by the roots of trees in preparing suitable soil for forest growth. Numerous investigators have found that the roots of trees frequently grow along channels of decaying or decayed roots of former generations. Here they meet little mechanical resistance, have an abundant supply of air, usually also plenty of water, and immediate access to the nutrients as rapidly as they are liberated in the process of decay. In some cases they seem to be of importance in directing the roots to the ground water. Coniferous roots develop especially well where the roots of hardwoods have made deep root channels.

That the adaptation of a species to its habitat is largely a matter of root development is a viewpoint that is being strongly supported by rapidly accumulating evidence. Open stands in forests are caused, not so much by intolerance to shade perhaps as by competition of widely spreading roots. AALTONEN (**1**) has reached the conclusion "that the space arrangement of those parts of the trees which are above the soil is mainly decided by their roots and the competition existing between them for the water and food in the ground." Other investigators have been led to a similar conclusion. BÜSGEN and MÜNCH (**6**) point out that the number, direction, and size of root branches express first of all adaptations to the environment, and that broad-crowned, deeply branched, isolated trees have therefore much larger masses of roots than those confined in dense woods. ADAMS (**2**) has shown that close spacing results in a marked change in the root form of jack pine, modifying its widely spreading root system into a vertical form with much branched, short, stubby roots.

The severest competition between individual trees takes place in

pure stands, such as the bur oak, since the roots tend not only to occupy the same layers of soil but to develop at about the same time. In mixed stands such as pine, birch, and spruce, competition among the roots has been shown to be much less severe than in an equally dense stand of a single species. This is due to the fact that the horizontal roots of these species occupy the soil at different levels. Thus in Europe an undergrowth of birch often thrives under spruce (**15**). Some interesting cases have been found where one species of tree has actually destroyed another by root competition, although the crowns did not touch. *Populus canadensis* has thus been replaced by *Elaeagnus angustifolia* and *Maclura aurantiaca* by *Ulmus campestris* (**17**).

From these data we may conclude that the small size, usually wide spacing, and very extensive root system of the bur oak are adaptations to a relatively dry soil and atmospheric conditions scarcely congenial to tree growth. Its successful competition against the grasses and shrubs is made possible by the rapid growth and deep penetration of the young root system and by its widely spreading habit which enormously increases the soil volume from which water may be secured to meet the needs of the growing top.

Summary

1. An ecotone between the grassland and the deciduous forest along the Missouri River occurs in southeastern Nebraska. The mean annual precipitation is 28–32 inches; humidity is relatively low, evaporation and wind movement are relatively high; the silt loam soils are deep but during drought contain only a small amount of water available for growth. Thus competition for water between the grasses and the invading shrubs and trees is great.

2. *Quercus macrocarpa* is the most xeric forest tree. Since the cessation of prairie fires it is invading the grassland either directly or in the wake of the *Rhus-Symphoricarpos-Corylus* chaparral.

3. The shrubs advance largely by means of rhizomes into the grassland. Their roots often extend outward and then upward under the grasses. Those of *Symphoricarpos* especially are so finely branched and abundant as to compete successfully with the excellently developed and extensive root systems of the plants of the prairie.

4. The oak seedling develops a deep taproot before the leaves are unfolded. The strong, finely branched taproot extends into moist soil 3–5 feet the first summer.

5. Mature trees 50–65 years old were 35–40 feet tall. 12–18 inches in basal diameter, and grew 10–40 feet apart in a pure stand.

6. The taproot gave rise to thirty or more large main branches, most of which arose in the first 2 feet of soil. It tapered rapidly and extended to a depth of 14 feet.

7. Most of the main branches, which varied from 1 to 7 inches in diameter, extended widely (20–60 feet) before turning downward. Some grew even deeper than the taproot. All branched repeatedly, and together they occupied a very large volume of soil.

8. Many branches of the main roots grew vertically downward 8–15 feet, each more or less resembling the taproot system of an oak sapling. Others extended obliquely or vertically upward and filled the surface soil with a mat of absorbing rootlets.

9. Ropelike roots, 0.5 inch or less in diameter, extended many feet without much change in thickness. A cordlike type, 3–5 mm. thick, was also abundant. A third type consisting of fine, much branched rootlets clothed the widely extending skeletal framework and furnished the bulk of the absorbing surface. Mycorrhizal mats were abundant.

10. The weight of the roots equaled that of the tops; the volume of the roots was about one-tenth less than that of the parts above ground.

11. Low water content of soil is compensated by a widely spreading, well branched root system. This may account for the wide spacing of the oak trees and the open forest canopy.

UNIVERSITY OF NEBRASKA
LINCOLN, NEB.

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A CYTOLOGICAL STUDY OF TULASNELLA

DONALD P. ROGERS

(WITH SEVENTY-NINE FIGURES)

Introduction

The fruiting layer of *Tulasnella* is a delicate bloom or film, waxy or mucilaginous, occurring most commonly on the lower surface of dead branches or logs or on the bodies of other fungi growing there. The fructification consists of a thin, very loose mat of hyphae, upon which the basidia are seated; the whole bears a marked resemblance to a tenuous *Corticium* or *Sebacina*. The basidium arises by the swelling of the terminal cell of a hyphal branch into a broadly pyriform body; from its summit are produced four oval sacs, each of which puts out from its apex a filament, and upon this bears a spore. The interest that the genus possesses lies in the organization of the basidium, the precise homologies of whose parts have not been conclusively demonstrated.

History

The TULASNES (**27**) first called attention to the peculiarities of the *Tulasnella* basidium. In 1872 they published a brief notice and a few figures which set forth its characters very clearly. Its points of resemblance to the basidia of both *Sebacina* and *Corticium* were so striking that these investigators noted it as an intermediate form and because of it withdrew an earlier statement that those two genera should be separated widely. They took their fungus to be *Corticium incarnatum* Fr. and allowed it to remain under that name.

In 1888, SCHROETER (**23**) published the description of his genus *Tulasnella*, "with basidia and spores formed in the same manner as in the fungus described by TULASNE." He agreed with the statement in the earlier paper concerning the intermediate position of the type. His new genus he listed as an "Anhang" to the Tremellinei, with the note that its inclusion within that group must remain doubtful. Later in the same year appeared PATOUILLARD'S account (**21**) of his genus *Prototremella*. He held that the similarity between

the basidium of this fungus and that of *Corticium* was only superficial. In *Prototremella* the basidial appendage, rather than the basal cell as in *Corticium*, is the "organ essential to the production of the spore"; and in *Prototremella* the spore germinates to form a secondary spore borne on a promycelium. This functioning of the parts of the true morphological basidium as separate basidia, in the older biological sense, and this germination of the spore by renovation, are the criteria of PATOUILLARD'S *Heterobasidii*, and so he placed his genus in that group, near *Tremella*.

In 1889, OLSEN (**4**) published his genus *Pachysterigma*, like *Prototremella* a synonym of SCHROETER'S name. The genus was placed lowest in BREFELD'S newly erected *Tomentellei*. Like the forms associated with it there, it was considered very primitive, since its basidia are not arranged in a regular hymenium. The basidium itself was regarded as an autobasidium.

The detailed cytological investigations upon two species of *Tulasnella* which JUEL (**16**) published in 1897 are not invalidated by his having described as a poroid tulasnellaceous form what was in reality a *Tulasnella* growing upon a *Poria* (**17**). He found that in *Tulasnella* the binucleate condition of the young basidium, karyogamy, and meiosis occur according to the scheme generally characteristic for basidia. In his *Muciporus* (that is, *Tulasnella*) *deliquescens* the first postmeiotic division takes place in the discharged spore, but in *T. thelephorea* it takes place in the basidial appendage. According to JUEL'S conception the latter body is the actual basidiospore, and the cell cut off and freed from its apex is a secondary spore or conidium. By the possession of sessile spores germinating in place, JUEL characterized his family Tulasnellaceae. He placed it, as a transitional group, between the Dacryomycetes (to him autobasidial) and the Hymenomycetes.

VON HÖHNEL and LITSCHAUER (**15**) in 1908 rejected JUEL'S interpretation and his family, and included *Tulasnella* among the Corticieae, "since its close relationship is unquestionable," and since "between the oval sterigmata of some species of *Tulasnella* and the subulate ones of *Corticium* all transitions occur." Likewise RAUNKIAER (**22**) rejected JUEL'S conclusions. He found his justification rather in direct criticism of JUEL'S arguments and observations.

Especially he questioned that any evidence had been presented to show that the inflated bodies upon the *Tulasnella* basal cell were in any degree separate entities. In the absence of such evidence he considered them not spores but only sterigmata, and the fungi in which they occur corticiaceous.

NEUHOFF, in his study of the heterobasidiomycetes (**20**), placed *Tulasnella* among them. According to his conception, the heterobasidium arises as a probasidium and is typically composed of two parts, a basal hypobasidium and one or more epibasidia which bear the sterigmata and spores. Thus in *Tulasnella* the pyriform basal cell (the hypobasidium) and its four appendages (epibasidia) together make up the basidium; the very slender filaments borne at the tips of the epibasidia are sterigmata; the spores upon them are the basidiospores. NEUHOFF placed *Tulasnella* in the line by which he held the Tremellaceae to have evolved from corticiaceous forms.

BOURDOT and GALZIN (**2**), like VON HÖHNEL and LITSCHAUER, noted that several species of *Corticium* show evidence of close relation to *Tulasnella*. Following PATOUILLARD, however, they retained the latter genus among the *Heterobasidii*.

Observations

Living material of five species of *Tulasnella* was available for the present study. *T. eichleriana* Bres., *T. fuscoviolacea* Bres., and *T. violea* (Quél.) Bourd. and Galz. were collected near Iowa City a number of times during the winter and early spring of 1930–31 and again in the late autumn of 1931. *T. tulasnei* (Pat.) Juel was collected at two places near Iowa City in November and December of 1931. *T. lactea* Bourd. and Galz. was collected once, at West Okoboji, Iowa, in July, 1931. Material of *T. lactea* was fixed in Allen's modified Bouin's fluid and of the other species in the weaker Flemming's fluid. All species were sectioned at 5 μ, and *T. tulasnei* at 8 μ also. Slides of all species were stained in Haidenhain's haematoxylin and counterstained with phloxine or erythrosin; in addition, slides of *T. violea* were stained in safranin, gentian violet, and orange G. Crush preparations of the first four species were used also; such preparations were first treated with a dilute solution of potassium hydroxide and then stained with phloxine. A portion of the type

collection of *T. anceps* Bres. and Syd. was available, in SYDOW's *Mycotheca germanica;* crush preparations and free-hand sections of it were studied.

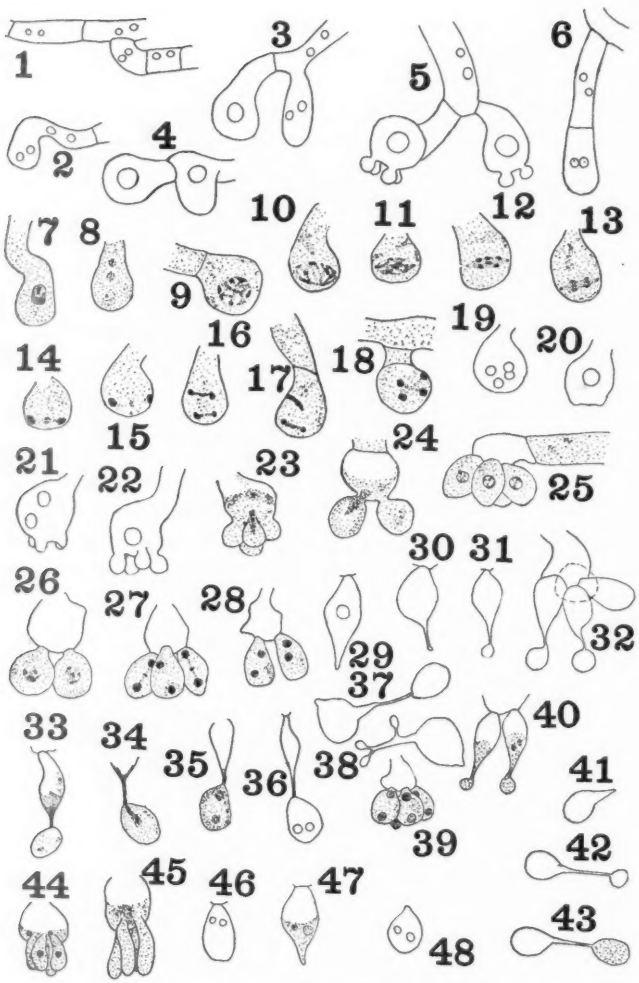
Material of *T. violea* appeared most favorable for cytological examination, and consequently received the most complete study. No more detailed account of the other species is given than seems necessary to supplement the observations upon the one species here regarded as typical.

TULASNELLA VIOLEA

The mycelium of *Tulasnella violea* penetrates the substratum to no great depth; occasional threads reach as deep as 300 μ, but the greater amount of mycelium lies within 50 μ of the surface. The superficial layer likewise is very thin; most commonly in the material examined it measures only 20–30 μ to the summits of the basidia, although in some areas it is as thick as 50 μ. The hyphae are usually 2–3 μ thick, and occasionally 4–5 μ; septa occur at frequent intervals; the diameter of the cells may be uniform or may decrease from the middle to the septa. The mycelium appears to be entirely binucleate (fig. 1).

The majority of the basidia arise directly from the horizontal hyphae of the receptacle rather than from vertical basidiophores. Most commonly the terminal cell of a superficial thread turns outward, so that its tip is perpendicular to the substratum (fig. 2); the tip, separated by a septum from the horizontal portion, becomes a probasidium. Somewhat less commonly the long axis of the basidium lies parallel to the substratum (figs. 4, 25), and the basidial appendages appear on the outer side rather than from the apex. Occasionally basidia are borne on simple vertical basidiophores (fig. 5). Subterminal basidia also occur; the penultimate cell itself may swell into a basidium (fig. 4); or the basidium may develop as a proliferation of some cell of the receptacle (figs. 3, 18). The diameter of the youngest cells recognizable as basidia is no greater than that of the hyphae that produce them.

The two primary basidial nuclei come to lie side by side in the developing cell. Here they remain without visible change until the probasidium has attained about half its maximum diameter. Just



FIGS. 1–48.*—FIGS. 1–38, *Tulasnella violea:* Fig. 1, hypha of receptacle. Fig. 2, end of horizontal hypha, the out-turned tip a very young probasidium; basidial dikaryon formed and cell swollen, but probasidium not yet cut off by septum. Fig. 3, terminal

* All figures were drawn with the aid of camera lucida, using Zeiss objective 90× and ocular 15×, except figures 69–72 and 74–79, drawn under 90× objective and 10× ocular, and figure 52, drawn without camera lucida. As reproduced, figures 69–72 and 74–79 are ×992, figure 52 about ×1300, and all others ×1390.

before fusion these nuclei increase somewhat in diameter (fig. 8); the fusion nucleus has about three times the original diameter of the primary nucleus. For a time after fusion the nucleus gives no evidence of further development; during this period the basidium increases considerably in size and becomes broadly pyriform (fig. 3).

Before division the nucleus is resolved into a spireme, considerably larger than the resting zygote, showing rather broad, deeply staining chromatin threads in a hyaline matrix (fig. 9). Presently this spireme gives evidence of polarity, becoming a broadly spindle-shaped structure reaching completely across the basidial cell near the summit (figs. 10, 11). From this is formed the much more slender mitotic spindle (figs. 12, 13). On the spindle a number of minute dark bodies—frequently six but sometimes, apparently,

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basidium formed and nuclear fusion in it completed; younger probasidium in process of formation on penultimate cell. Fig. 4, terminal probasidium with long axis parallel to receptacle; penultimate cell transformed into probasidium. Fig. 5, two basidia borne on vertical basidiophore. Fig. 6, probasidium occurring as terminal member of vertical hypha. Figs. 7, 8, dikaryons in process of fusion. Fig. 9, spireme. Figs. 10, 11, later spiremes possessing polarity. Figs. 12, 13, spindles of first meiotic division. Figs. 14, 15, later stages of first division; deeply stained body on spindle between reorganizing nuclei. Fig. 16, second meiotic division; spindles parallel. Fig. 17, second division; spindles crossed; left end of lower spindle and right end of upper in higher plane; dark body at equator of lower spindle. Fig. 18, later stage of second division; areas occupied by spindles still staining deeply; spindles crossed. Fig. 19, four-nucleate basidium still lacking appendages. Fig. 20, basidium with appendages barely apparent; zygote nucleus undivided. Fig. 21, basidium with zygote nucleus once divided. Fig. 22, basidium with conspicuous appendages; zygote nucleus undivided. Figs. 23, 24, basidia partially drained of protoplasm; nuclei in passage to appendages. Fig. 25, appendages cut off from exhausted basal cell. Fig. 26, spireme in appendages; basidium in section showing convexity of septa at bases of appendages. Fig. 27, late stage of third division in (two) appendages; deeply staining body at equator of spindles; division in one appendage completed. Fig. 28, section of basidium with binucleate appendages; basal cell partially collapsed. Fig. 29, uninucleate appendage in process of putting out filament. Figs. 30–32, early stages in spore formation. Fig. 33, appendage nearly drained of protoplasm by spore. Fig. 34, nuclei in migration into spore; apparently first nucleus near middle of spore and second partly in filament, partly emergent. Fig. 35, immature spore, lateral upon filament, nuclei in opposite ends. Fig. 36, mature spore. Fig. 37, spore bearing nearly mature secondary spore. Fig. 38, spore bearing conidia. FIGS. 39–43, *T. eichleriana:* Fig. 39, basidium with binucleate appendages cut off by septa. Fig. 40, appendages bearing young spores. Figs. 41–43, stages in production of secondary spore. FIGS. 44–48, *T. lactea:* Fig. 44, young appendages, two with nuclei, upon partly exhausted basal cell. Fig. 45, basidium with longer, narrow appendages. Figs. 46, 47, binucleate appendages, with basal septum. Fig. 48, binucleate spore.

fewer or as many as eight—can be made out; these are to be interpreted as the chromosomes. There often appears to be a very minute black point at one or both poles; whether a centrosome is present there is not certain. The telophase of this division shows two deeply staining bodies, the reorganizing nuclei, lying close to the walls of the cell, with a dark line between them having a darker body midway between the two ends (figs. 14, 15). The newly formed daughter nuclei move somewhat away from the walls.

Apparently the formation of basidial appendages is not correlated with nuclear change; the first and even the second meiotic division may occur in a probasidium which shows no sign of developing appendages (figs. 10–19); or the zygote nucleus may still remain in a resting condition when the proliferations have reached a length one-third as great as that of the basal cell (fig. 22). The first indication of the rise of the appendages is little more than a slight flattening of the summit of the probasidium (fig 20). Presently small knobs are formed (fig. 21). These swell distally without greatly increasing in diameter at the base, becoming short-stalked globes (fig. 22) and then, through elongation, pyriform bodies (fig. 23).

The nuclear spindles in the second meiotic division are transverse, either crossed at different levels near the summit of the basal cell (figs. 17, 18) or lying approximately parallel (fig. 16). The four nuclei formed lie near the apex of the basal cell until the appendages have attained considerable size (a length somewhat greater than half that of the basal cell), and by their increase in volume have partially exhausted the latter structure. The four nuclei thus come to be placed near the base of the appendages in a body of protoplasm that fills only the upper portion of the cell, most of the lower portion being occupied by a large vacuole (fig. 23). Finally the nuclei pass into the appendages, becoming somewhat elongated and more deeply stainable in so doing (figs. 23, 24); with them goes most of the remaining protoplasm. Apparently a film of protoplasm is left in the basal cell, since the latter does not collapse for some time (figs. 25–27).

As soon as the migration of the protoplasm into the appendages has been completed, septa are formed across the communicating passages, cutting off the appendages from the basal cell (figs. 25–28).

These septa can be made out clearly in the haematoxylin-phloxine preparations, the counterstain being strong enough to bring out the wall as a sharp pink line; they are even more marked in the preparations made with the Flemming triple stain. They are of course most evident in basidia which have been sectioned longitudinally (fig. 28). In the appendages thus cut off as separate cells a third mitosis then occurs. The spindles lie longitudinally; a late stage shows the same dark strand connecting the reorganizing nuclei, the same dark line in the equatorial position (fig. 27) that appeared in the meiotic mitoses. The daughter nuclei here formed approach each other somewhat; they may come to lie side by side in the cell or remain one above the other.

This division, like the earlier ones, is not correlated with change in the form of the cell (figs. 28, 29). But soon after mitosis has been completed the appendage has become narrower at the distal end and thus broadly spindle-shaped (fig. 29); a filament arises from the tapering apex (fig. 30). Elongation continues until the cell has reached a length half again as great as that of the probasidium; narrowing of the outer end continues, so that the appendage may become nearly conical (fig 32). The basal cell by this time commonly has commenced to collapse; presently it has so far lost its earlier form as to become only a folded membrane lying at the base of the four walled-off appendages.

The spore first appears as a minute lateral swelling on the tip of the appendage (fig. 30). By the time it has attained half its final diameter the appendage has been so far exhausted of its protoplasm as to have much of the basal part filled with a large vacuole; the narrowing of the distal portion has progressed further toward the base (fig. 33). Presently the two nuclei pass into the spore, becoming much elongated and deeply stainable throughout as they pass through the very slender terminal portion of the filament (fig. 34). At first they lie one above the other (fig. 35); but later, and apparently always in the discharged spore, side by side near the blunt end (fig. 36). The mature spore is borne somewhat laterally upon the tip of the filament, the insertion of the latter being just beside the well developed apiculus of the spore. Living material studied in a moist chamber showed a drop of water held between the apiculus and

the filament, as figured for *T. fuscoviolacea* (fig. 52). This relation of filament and spore being precisely that given by BULLER (**9**, p. 498) as characterizing the sterigma and spore of Basidiomycetes whose spores are forcibly discharged, it must appear highly probable that such discharge occurs here; but none was observed.

The spore in germination produces most commonly a secondary spore, smaller, similar in form, borne at the tip of a short promycelium (fig. 37). Often, however, germination is by the production of numerous minute oblong conidia, about 1 μ long, borne on a slender, simple or branched filament (fig. 38).

TULASNELLA EICHLERIANA

In all characters except size, *Tulasnella eichleriana* is identical with the preceding species. The septum dividing appendage from basal cell is as clear as in the larger species, and mitosis within the appendage is as invariable (fig. 39). Only the one type of germination, by the production of a secondary spore, was observed (figs. 41–43).

TULASNELLA FUSCOVIOLACEA

In basidial cytology *Tulasnella fuscoviolacea* is identical with the preceding species, and in structure very similar. Here also the first postmeiotic division occurs in the appendages, after they have been separated by a wall from the basal cell (fig. 51). It differs from the two very similar species in the somewhat more globose form of the probasidium and appendages, in the allantoid spores, and in the greater size of all its parts. Material of two collections which seem to belong here shows fairly frequent clamp connections at the bases of the basidia (especially conspicuous on the young probasidia, fig. 49), and occasional clamp connections throughout the superficial mycelium (fig. 53). The occasional separation from the basal cell and subsequent functioning in spore production of the appendages, noted in this species by MARTIN (**19**) as in *T. deliquescens* by JUEL, could never be surely demonstrated from material touched by the sectioning knife; the regular collapse and disintegration of the basal cell before the formation of the spore indicates the probability of such an occurrence. In living material studied in a moist chamber the spores are borne in a lateral position upon their filaments; a

small, persistent water drop is held between apiculus and filament (fig. 52). Spore germination is by the production of a short promycelium, lateral or apical (figs. 55, 56), upon whose attenuated tip a secondary spore is borne. No conidia were observed.

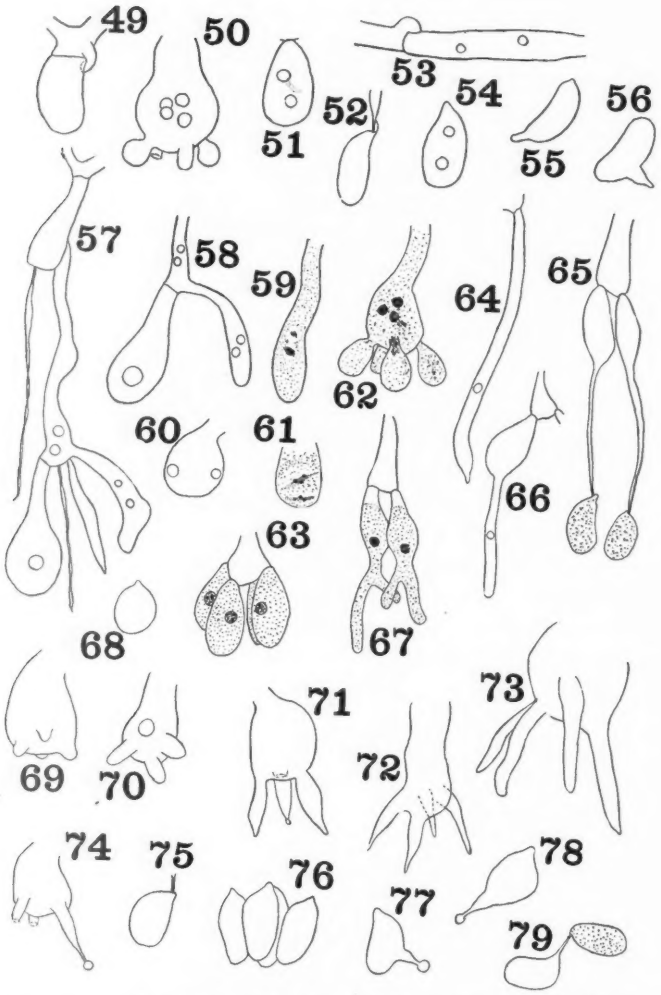
Tulasnella lactea

The cytological development of *Tulasnella lactea* corresponds to that of the preceding species in all points, including mitosis in the appendages and formation of septa between basal cell and appendages (figs. 46, 47). The latter are grouped much more closely together than in the other species, upon the very summit of the basal cell (fig. 44). The texture and structure of the receptacle are notably different; it is composed of an intertwining mass, 30–50 μ in depth, of thick, irregular, vertical hyphae; on the tips of these threads are borne the small basidia.

Tulasnella tulasnei

In macroscopic and microscopic appearance *Tulasnella tulasnei* is quite distinct from the other species collected. It forms a continuous gelatinous-waxy layer, in places minutely tuberculate, in places smooth, much more strongly developed than the delicate bloomlike fructifications of the smaller forms. This layer deliquesces almost immediately upon maturity of the basidia, becoming a gelatinous slime, slightly undulate, finally perfectly hyaline.

The basidia are borne upon heavy vertical hyphae, commonly a number of them in a cluster upon one strong axis. Borne with the basidia upon such basidiophores, and upon the receptacle among them, are slender paraphyses (fig. 57) whose early deliquescence gives to the fruiting layer its gelatinous texture. The youngest basidia are clavate bodies (figs. 58, 59), often 20 μ long. These swell distally to form a broader portion (fig. 57) in which karyogamy and meiosis occur. The appendages arise as globose protrusions and enlarge to form oval bodies, which are then walled off at their bases (figs. 62, 63). In this species there is no third mitosis in the basidial appendage. Soon after formation of the septum there is thrust out from the appendage a filament which elongates greatly, often reaching a length greater than 20 μ before forming its spore (fig. 65). This



FIGS. 49–79.—FIGS. 49–56, *Tulasnella fuscoviolacea:* Fig. 49, probasidium with clamp connection at base. Fig. 50, four-nucleate basidium. Fig. 51, binucleate appendage with septum across base. Fig. 52, living spore upon filament, borne laterally, with water drop between apiculus and filament. Fig. 53, hypha of receptacle, with clamp connection. Fig. 54, binucleate spore. Figs. 55, 56, spores with promycelia. FIGS. 57–68, *T. tulasnei:* Fig. 57, vertical basidiophore bearing probasidium (at left), two half-

great length of filament appears to be an adaptation to the presence of the thick gelatinous matrix about the basidia. Often the basal portion of the appendage remains slender or becomes contracted, so that the diameter of the organ is about the same throughout its length (fig. 64). Frequent abnormalities found in this species, such as greatly attenuated, hypha-like appendages, often attaining a length of 100 μ and possessing protoplasm in only the distal third or quarter, or branching appendages (fig. 67), equally common, are also to be attributed to the gelatinous matrix. Spore germination is by secondary spore or by direct production of mycelium.

Tulasnella anceps

The mycelium of the fungus described as *Tulasnella anceps* is much like that of *T. violea* in general habit: loose, branching often at right angles, composed of short, thick cells. The basidia arise, like those of *T. violea*, as subglobose or pyriform cells, usually terminal upon the hyphae of the receptacle. From the summit of such a cell are thrust out commonly four rounded knobs (fig. 69) which elongate to form thick appendages of uniform diameter (fig. 70). The ends of these appendages then taper to a point, and often the diameter near the middle increases somewhat, before the formation of spores begins (figs. 71, 72). The mature basidium agrees in all details with the original description of Bresadola and Sydow (**6**). Obviously no cytological observations were possible upon the material at hand; crush preparations showed clearly that no septa are formed at the bases of the basidial appendages (figs. 71–74). The spores regularly germinate by the formation of a similar secondary spore borne on a

disintegrated paraphyses, and young paraphysis not yet deliquescent; binucleate proliferation (at right), apparently a continuation of basidiophore. Fig. 58, young probasidia. Fig. 59, dikaryon in process of fusion. Fig. 60, probasidium after first division of zygote nucleus. Fig. 61, crossed spindles of second division; left end of lower spindle and right end of upper in higher plane. Fig. 62, basidium with nucleus in migration into appendage. Fig. 63, uninucleate appendages cut off by septum from exhausted basal cell. Fig. 64, elongated, slender appendage, uninucleate. Fig. 65, spores in place upon filaments of emptied appendages. Fig. 66, appendage with its single nucleus lying in filament. Fig. 67, appendages, uninucleate, with forked filaments. Fig. 68, spore. Figs. 69–79, *T. anceps:* Figs. 69–74, basidia in various stages; appendages all lacking basal septa. Fig. 75, spore upon sterigma. Fig. 76, four spores clumped together. Figs. 77–79, spores in process of forming secondary spores.

short promycelium (figs. 77–79). Frequently the spores are clustered, lying parallel and in contact; such a group (fig. 76), especially when some of the spores have formed promycelia, greatly resembles a group of four tulasnellaceous basidial appendages; but no basal cell can be observed attached to them, and what appears to be the tapering distal end of an appendage proves to be the apiculus of a spore. Such grouping might result from the spores being borne close together on curving sterigmata, in the manner figured by BURT (**11**, p. 296) for *Corticium vagum*.

Discussion

JUEL'S interpretation of the basidial apparatus of *Tulasnella* is perhaps the one most widely accepted. He gives the following as his grounds for regarding as basidiospores the oval bodies upon the basal cell:

(*a*) At a certain phase of their development—just before their germination—the form of these bodies is wholly sporelike.

(*b*) These bodies can fall from the basidium and germinate, although this occurs only exceptionally.

(*c*) The tubes that are attached to the upper parts of the sterigmata [!] vary considerably in length, even on the same basidium. . . . . This applies less well to sterigmata, since they should be about equally long. But germ-tubes or promycelia have mostly no determinate length.

(*d*) These germ-tubes can at times arise at the base of the bodies interpreted by me as spores, and even exceptionally there can be two upon the same spore. Such an occurrence can hardly be explained by the earlier interpretation.

(*e*) The anomalous branching tubes figured are very strange if one interprets them as sterigmata, but according to my interpretation not at all notable.

(*f*) Cross-walls in the germ-tubes of germinating spores are known also in other fungi, and the structures figured can pass very well for germ-tubes, but less well for sterigmata.

(*g*) In *Muciporus corticola* [that is, *Tulasnella thelephorea*] each of the four nuclei found in the basidium divides as soon as it has passed from the basidium into one of the oval bodies. Since we interpret these bodies as spores, this nuclear division is not especially noteworthy. In a sterigma, however, that forms only a canal for the transfer of the basidial content to the spores, one would not expect nuclear division.

NEUHOFF'S argument is based upon the resemblances of the *Tulasnella* basidium to that characterizing the Tremellaceae. Formation of a globose probasidium from which after a time are thrust

out commonly four thick tubes; production of spores after the basidial protoplast has been divided by walls into independent daughter protoplasts; germination of the spore by the formation on a promycelium of a similar secondary spore—these are tremellaceous characters, and tulasnellaceous as well. According to NEUHOFF'S conception, that structure of the heterobasidium which the earlier workers called a sterigma is not homologous with the sterigma of the autobasidium; rather the basidium proper among the Tremellaceae possesses more or less the form of a hand and fingers. For the definitive sterigma, borne on the tip of the basidial extension, the epibasidium, NEUHOFF gives as a criterion that a migrating nucleus in passing through a sterigma assumes an elongated form and gives a staining reaction like that of chromosomes. This criterion is subject to the criticism that it may be only a response of the nucleus to the spatial relation, to be expected wherever a nucleus passes through a narrow opening (**7**, p. 9). But this very slenderness sets off the sterigma as a morphological unit from the epibasidium; and the criterion of function given by BULLER (**8**, p. 31), that "the typical sterigma . . . . is to be regarded as an organ for the violent discharge of the spore," serves as a further indication of the validity of NEUHOFF'S distinction.

The arguments which JUEL presents against the homologizing of the *Tulasnella* basidial appendages with sterigmata are not antagonistic to NEUHOFF'S conception. The shape of the appendage (*a*, of JUEL'S points) when it has reached its complete development and formed its spore is as much like that of the *Tremella* epibasidium as at an earlier stage it is sporelike. Its germination when separated from the basal cell (*b*) is essentially only what occurs in *Tremella;* in neither are the spores formed until the basidial protoplast is divided by walls into independent daughter protoplasts, each of which is then capable of producing a spore—the *Tulasnella* appendage even though separated from the exhausted basal cell, the *Tremella* basidial segment even though, as often occurs, the basidium may have become separated from the exhausted hypha on which it was borne. Somewhat similarly, in certain Ustilagineae (**28**) and in *Septobasidium retiforme* (**13**, p. 127), the epibasidium—a structure somewhat different, but like the appendage of *Tulasnella* in being only the

upper portion of the basidium—may form spores when separated from the hypobasidium. Variation in length (*c*) is the rule with *Tremella* epibasidia also (**24,** p. 195, pl. 10, figs. 9, 14, 26). Basal insertion of the filament, and the presence of two filaments (one basal), upon a single oval body (*d*), as figured by JUEL, are apparently geotropic responses to altered position of the basidium, and of no more morphological significance than what JUEL refers to as the lateral insertion of appendages, occurring wherever the long axis of a basidium lies horizontally. Branching of the apical filament (*e*) as figured by JUEL is no branching at all, but the lateral insertion of the sterigma; it occurs in *Tremella* (**3,** pl. 7, fig. 14 (3); **24,** pl. 10, fig. 17, pl. 11, fig. 1). True branching does occur, however, in *Tulasnella* and in *Tremella* (**18,** pl. 6, fig. 14 *c;* **24,** p. 196, pl. 12, fig. 9). Cross walls in the filament (*f*) may be accounted for by the development under water which JUEL gives as the cause of another abnormality of the same specimen, or perhaps even better by the gelatinous matrix in which the basidia lie imbedded; they have been noted in *Tremella* (**24,** pl. 10, fig. 16). Such hypha-like characters as septation, branching, and indefinite elongation have been observed by the writer to occur in the epibasidia of somewhat deliquescent specimens of *Tremella lutescens*. Mitosis within the appendage (*g*) is not the impossible behavior for an extension of the basidial cell that it would be for a sterigma.

Some of this has been said by NEUHOFF, and all of it is implied in his theory. His conception of the epibasidium provides for all the characteristics and abnormalities of the basidial appendages of *Tulasnella*, including their great similarities to the comparable organs of *Tremella;* it appears certain that this structure of *Tulasnella* is an actual epibasidium. The Tulasnellaceae are to be regarded as nearly related to the Tremellaceae, the two families being separated through the difference in position of the septa by which the basidial protoplast is finally divided. There appears no reason for questioning the assumption that the holobasidium (the *Tulasnella* basidium being such for this purpose) is nearer the ascus in morphology and phylogeny than the phragmobasidium. It must therefore be an acceptable hypothesis that the Tulasnellaceae, or forms very similar, are the ancestors of the Tremellaceae. The tremellaceous basidium may be taken to have arisen from the tulasnellaceous

basidium by the formation earlier in basidial ontogeny of the septa by which the basidial protoplast is divided.

The autobasidiomycete affinities of *Tulasnella*, perhaps equally strong, have been recognized in some degree by all investigators of this genus. HÖHNEL and LITSCHAUER gave them greatest weight, but failed to publish the list of transitional forms which justified to them the inclusion of *Tulasnella* among the Corticieae. However, such a series can be constructed, of *Corticium* species whose sterigmata show progressively greater length and thickness, in the final forms of the series resembling the greatly inflated epibasidia of *Tulasnella*. BOURDOT and GALZIN note as extreme forms, very close to *Tulasnella*, *Corticium flavescens* (Bon.) Massee, *C. sterigmaticum* Bourd., and *C. vagum* Berk. and Curt.

The fungus described as *Tulasnella anceps* Bres. and Syd. may be taken to be transitional. Its relatively enormous so-called sterigmata are usually broadest at the base, but frequently somewhat the widest midway between base and spore, being thus spindle-shaped. In their earlier stages they are blunt protuberances quite like the young epibasidia of typical *Tulasnella* species. But they never attain the width or the volume of the spore produced at their apex; they are never walled off from the basidial cell; they cannot be the organs "essential to the production of the spore"; they are not tulasnellaceous epibasidia, although very close to them. The formation of the basidia directly upon the loose weft of hyphae that clings to the substratum is like that of the less gelatinous *Tulasnella* forms: the germination of the spore by the production of a secondary spore borne on a promycelium is a heterobasidiomycete character.[1]

[1] The taxonomic standing of *Tulasnella anceps* remains in question. BOURDET and GALZIN list it as a synonym of *Corticium vagum* Berk. and Curt. Its basidia agree well with those of the latter species, and the frequent clumping of its spores would be most easily brought about by their being borne in the manner shown by BURT for *C. vagum*. But in the somewhat meager material of *C. vagum* available for comparison, no spores show the germination by renovation characteristic of SYDOW's specimens. However, the *C. vagum* studied, growing as it did on potato stems, would have been placed by BOURDOT and GALZIN in *C. solani* (Prill. and Delacr.) Bourd. and Galz. The problem is complicated by this separation, which BURT (**11**, p. 295) does not recognize. In any event, the fungus distributed by SYDOW is not a *Tulasnella*, and cannot be taken, as suggested by CLEMENTS and SHEAR (**12**), to be the type of the genus. BOURDOT and GALZIN identify SCHROETER's species with *T. violea* (Quél.) Bourd. and Galz.; since the identity of the TULASNES' fungus cannot certainly be determined, *T. violea*, published in 1882, must be the type.

The nature of this fungus, and of other similar species among the simplest corticiums (one other whose spores germinate by renovation), suggests the further hypothesis that *Tulasnella* is the ancestral form of *Corticium*, and through it, of the autobasidiomycetes. The autobasidium may be taken to have arisen by the loss of the septum across the base of the epibasidium and subsequent telescoping of the epibasidium, the sterigma finally coming to be seated upon the basal cell.

Although JUEL's arguments do not demonstrate the homology of the *Tulasnella* basidial appendages with basidiospores, yet they are valid enough as indications of the sporoid nature of the bodies in question. The existence of a septum across the base of the epibasidia, a structure which JUEL failed to observe (**16**, plate), and the supposed absence of which is the one basis of RAUNKIAER'S criticism of JUEL'S theory, is an even more cogent argument here. In discussing this family, GÄUMANN and DODGE (**14**) state:

> If the basidium is conceived as developing from an ascus by gradual exogenous spore production, perhaps *Tulasnella* represents a transitional stage where the spore mass, without secreting a wall about itself, is pushing out of the gonotocont without having reached the stage of a separate entity before it germinates. . . . . On the other hand, such a conception would indicate that it had not yet reached a suitable mechanism for spore discharge, having lost that of the ascus without attaining that of the basidiospore.

This suggestion embodies just such a conception as will account for the sporoid characters of the *Tulasnella* epibasidium. It falls short of adequacy in accepting that the body in question has not "reached the stage of a separate entity," in denying to the fungus "a suitable mechanism for spore discharge," and in homologizing the oval bodies upon the *Tulasnella* basidium with basidiospores.

The cytological homologies between basidium and ascus, and between basidiomycete dikaryon hypha and ascogenous hypha, make derivation from the Ascomycetes the one acceptable theory of the rise of the Basidiomycetes. Such derivation may well be taken to be from such a form as *Ascocorticium* (**5**, pp. 145, 146), whose general structure resembles that of the simpler resupinate basidiomycetes. If in some such fungus ascospores, in place of being discharged in the jet, were to be merely thrust out in pockets of the acsus wall, a

transitional form leading toward the basidium would there be achieved. If then the ascopore protoplast, its wall failing of formation, were to be surrounded rather by the ascus wall of the pocket and a septum formed across its base, the body thus produced, containing the haploid nucleus, although not actually a spore, would be capable of exhibiting such sporoid characters as JUEL lists; it would be just such a body as the *Tulasnella* epibasidium. Ascospores are known to germinate by the formation of conidia on a short promycelium (**25,** p. 177; **26,** pl. 16, fig. 19); such a conidium produced in the germination of the undischarged ascospore protoplast might well be taken to be the ancestor of the basidiospore. A similar interpretation was advanced by BESSEY (**1**), who, homologizing the segments of the rust basidium with ascospores, termed the basidiospores secondary spores. Whether for a time in the phylogenetic history of these forms the walled-off appendages may have fallen from the basal cell, germinating subsequently, or whether germination upon the basal cell came at once, at present cannot even be conjectured and seems not essential to the hypothesis. Similarly, there are not sufficient grounds for indicating the relations among the 5–8-spored species of *Tulasnella*, the 4-spored species with mitosis in the epibasidium, and the 4-spored species without such mitosis. The postmeiotic division occurring before basidiospore production may represent the delayed third division of the zygote nucleus—a conception admissible here, since the present *Tulasnella* epibasidium is certainly not an ascospore—or a step in ascospore germination.

Summary

1. Following karyogamy and the two meiotic divisions, the probasidium of *Tulasnella* produces from its summit commonly four inflated epibasidia; into these migrate the nuclei and protoplasm of the basal cell; when the latter has been exhausted the epibasidia are separated from it by septa across their bases. In *T. violea*, *T. fuscoviolacea*, *T. eichleriana*, and *T. lactea* a third mitosis occurs in the epibasidia; following this each epibasidium elongates and upon a filament produced from its apex bears a binucleate basidiospore. In *T. tulasnei* there is no mitosis in the epibasidia.

2. Similarities existing between the *Tulasnella* basidium and that of the Tremellaceae argue the close relation of the two types; the tremellaceous basidium may be regarded as having evolved from the tulasnellaceous by the formation of basidial septa at an earlier stage in basidial ontogeny. Similarly the existence of transitional forms (among them the fungus described as *T. anceps*) argues the close relation of the *Tulasnella* basidium to that of *Corticium;* the autobasidium may be regarded as having evolved from the tulasnellaceous type by loss of the epibasidium. The sporoid characters of the *Tulasnella* epibasidium strongly suggest its evolution from the ascospore; consequently the epibasidium may be taken as the true homologue among the basidiomycetes of the ascospore, and the basidiospore as the homologue rather of an ascospore germination-conidium.

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UNIVERSITY OF IOWA
IOWA CITY, IOWA

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ECOLOGICAL RELATIONSHIPS OF PINUS SABINIANA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 438

GEORGE W. GRAVES

(WITH FOURTEEN FIGURES)

Introduction

As one ascends the Sierra Nevada from the west, the most conspicuous feature of the landscape of the foothills and the lower mountain slopes at about the 600-foot level is *Pinus sabiniana*, known locally as the Digger pine. It is scraggly and irregular in growth, attaining a height of from 40 to 90 feet, and frequently dichotomously branched. This species occurs in open stands, reaching in more favorable situations the status of a thin open forest. Its zonal distribution is interesting, for it occurs only between 600 and 4000 feet elevation, with a peculiar gap in its range south of the Kings River. It is widely distributed in California on the interior coast ranges and the western slope of the Sierra Nevada.

The ecology of the area under discussion has been studied by various investigators. JEPSON (5) discusses the geographical distribution of the seed plants in California. COOPER (3), in his extensive treatment of the broad sclerophyll vegetation of the same state, considers the Sierra Nevada woodlands and chaparral although studying more in detail the coastal and southern sclerophylls. KLYVER (6) has recently mapped the plant associations in a transect 7 miles wide by 80 miles long across the Sierra Nevada. This transect covers the northern edge of the area discussed in this paper. The Forest Service is studying the woodlands and chaparral in connection with other vegetation types in relation to fire. Papers by SHOW and KOTOK (11, 12) treat this phase of the subject.

Species of the woodland and chaparral in this and other parts of the state are considered in papers by CANNON (1, 2), PLUMMER (8), MILLER (7), and STERLING (10).

Investigation

Vegetation types

The vegetation types are arranged in the order in which they are encountered in ascending the mountains from the San Joaquin Valley floor. A more detailed list of species is given under the discussion of succession.

Grassland.—The San Joaquin Valley floor is largely under cultivation, either by dry farming or by irrigation, except in those areas where an accumulation of alkali makes cultivation impossible or too expensive. The climatic conditions prevailing and what relict evidence can be obtained indicate that the predominant association was of a bunch grass type dominated by *Stipa pulchra* which is now restricted to small isolated areas. Where land is not cultivated or where cultivation has been abandoned, the original grasses have been largely replaced by a variety of introduced grasses and herbs.

Tree species occur only along the water courses or the floodplains of the Kings and San Joaquin rivers. These rivers have built up deltas of considerable size, and in overflow areas and along modern irrigation ditches hydric associations of *Typha*, *Scirpus*, *Anemopsis*, etc., are found. Habitats high in salt content are dominated by *Distichlis*, *Sporobolus*, *Salicornia*, *Allenrolfea*, *Sarcobatus*, etc.

Oak savanna.—In contact with the grassland of the valley floor and the frontal foothills is the oak savanna. It begins just inside the frontal foothills at an elevation of about 500 feet. In its lower portion it is open and parklike, with the trees at wide intervals. These are limited to *Quercus douglasii*[1] and *Q. wislizenii*, and layer shrubs as *Ceanothus cuneatus* and *Arctostaphylos mariposa* are restricted in distribution. In isolated rocky outcrops close to the crest of the frontal foothills small associations of *Rhamnus californica* are found.

Throughout the woodland zone there is a great uniformity of species. The grasses and herbaceous plants bloom early, forming in many cases aspect societies of great beauty. The period of bloom is very short and during the rest of the year the hillsides are dry and brown, except for the grey-green of the sclerophyllous plants.

[1] Plant names are from Jepson (**5**).

The following species are distributed throughout the area of the oak savanna:

Shrubs and trees of oak savanna

STREAM BANKS	OTHER SITUATIONS
Cephalanthus occidentalis	Arctostaphylos mariposa
Platanus racemosa	Ceanothus cuneatus
Populus fremontii	Lotus scoparius
Salix nigra	Nicotiana glauca
S. sessilifolia var. hindsiana	Quercus douglasii
	Q. wislizenii
	Rhamnus californica

Grasses and herbs forming conspicuous societies

Amsinckia douglasiana	Layia platyglossa
Avena barbata	Lupinus albifrons
A. fatua	L. densiflorus var. lacteus
Baeria chrysostoma	L. formosus
Brodiaea capitata	Madia elegans
B. ixioides	Montia perfoliata
B. laxa	Phacelia californica
Calandrinia caulescens	Plagiobothrys canescens
Gilia capitata	P. nothofulvus
Godetia amoena	Platystemon californicus
Heliotropium curassavicum	Trifolium tridentatum

Grasses and herbs of general distribution

Asclepias californica	Gilia tricolor
A. cordifolia	Hordeum murinum
Athysanus unilateralis	Juncus balticus
Brodiaea coronaria	J. mexicanus
B. hyacinthina	Lathyrus nevadensis
B. volubilis	L. sulphureus
Bromus hordeaceus	Layia glandulosa
B. rubens	Lepidium nitidum
B. tectorum	Limnanthes douglasii
Calochortus luteus	Linanthus bolanderi
C. venustus	L. dichotomus
Calyptridium umbellatum	L. serrulatus

Chaenactis glabriuscula
Cirsium occidentale var. coulteri
Collinsia bicolor
Cotyledon laxa
Cryptantha flaccida
Delphinium decorum
Diplacus longiflorus
Eschscholtzia californica
E. lobbii
Festuca megalura
Galium aparine
Gilia gilioides
Lotus subpinnatus
Mentzelia lindleyi
Mimulus bicolor
Mimulus guttatus
Monardella candicans
Plantago erecta
Raphanus sativus
Rumex acetosella
R. crispus
Salvia columbariae
Sanicula bipinnatifida
Senecio douglasii
Silene gallica
Silybum marianum
Sisyrinchium bellum
Solanum xantii
Stellaria media

DIGGER PINE-OAK WOODLAND.—At an elevation of about 600 feet the oak savana woodland merges into the Digger pine-oak woodland. It differs from the vegetation just below it in the conspicuousness of *Pinus sabiniana*. Although the grasses and herbs are an important phase, the trees in many cases form an open sclerophyll forest. Associations of layer shrubs such as *Ceanothus, Arctostaphylos mariposa*, *Rhus diversiloba*, and *R. trilobata* are common, as are treelike shrubs such as the deciduous *Aesculus californica* and *Cercis occidentalis*. In many cases this woodland becomes a brushland as it grades into hard chaparral just above it.

In addition to the species listed for the oak savanna, the following are characteristic of the Digger pine-oak woodland.

Shrubs and trees of the Digger pine-oak woodland

STREAM BANKS	OTHER SITUATIONS
Alnus rhombifolia	Aesculus californica
Fraxinus dipetala	Berberis californica
F. oregona	Ceanothus divaricatus
Salix lasiandra	Cercis occidentalis
	Cercocarpus betuloides
	Pinus sabiniana

Shrubs and trees of the Digger pine-oak woodland—Continued

Vines	Other situations
Echinocystis macrocarpa	Rhamnus crocea
Lonicera interrupta	Rhus diversiloba
Vitis californica	R. trilobata
	Sambucus velutina

Grasses and herbs of general distribution

Allium spp.	Nemophila menziesii
Clarkia elegans	Orthocarpus erianthus
Dodecatheon hendersonii	Panicum hirtecaule
Eleocharis palustris	Potentilla glandulosa
Galium trifidum	Saxifraga virginiensis var. californica
Hemizonia wrightii	
Lotus scoparius	Scrophularia californica
Lupinus stiversi	Thysanocarpus curvipes
Nemophila maculata	Trifolium ciliatum

Chaparral.—The term chaparral is used in various ways. Jepson (5) calls the type of chaparral occurring here the "hard" type to distinguish it from the "soft" type which is a shrub formation found in the Transition Zone. The practically pure stands of *Adenostoma fasciculatum* which cover large areas in southern California, on the Coast Range and on the foothills of the Sierra Nevada farther north are also called chaparral. *Adenostoma*, however, has not been collected in this area.

Hard chaparral is comparable with the maquis of the Mediterranean region. It is a mixed formation, with many tree species adapted to the xerophytic conditions, and has undoubtedly been greatly influenced in extent and structure by fire. Since the term chaparral has become established it will be used in this study to denote this particular type.

The chaparral then, ranging in elevation from 2000 to 4000 feet in this area, is a dense, almost impenetrable thicket, woody and evergreen except for a few deciduous forms, the most notable being the distinctive *Aesculus californica*. *Aesculus* adapts itself to its xerophytic situation by an early production of leaves and flowers. By the middle of June its leaves are dry and brown and the trees appear as brown blotches on the hillsides.

The most common genera of the chaparral are *Quercus*, *Ceanothus*, *Arctostaphylos*, *Umbellularia*, *Fremontia*, and *Aesculus*, with frequent specimens of *Pinus sabiniana*.

Because of the density of the stand, grasses and herbs occur sparingly in the mature chaparral. A list of species that have invaded the chaparral following fire is given under the discussion of succession in relation to fire.

Shrubs and trees of the chaparral

Aesculus californica	Prunus subcordata
Arctostaphylos mariposa	Ptelea baldwinii var. crenulata
Berberis californica	Quercus chrysolepis
Calycanthus occidentalis	Q. douglasii
Carpenteria californica	Q. wislizenii
Ceanothus cuneatus	Umbellularia californica
C. divaricatus	Rhamnus crocea
C. integerrimus	R. californica
Cercis occidentalis	Rhus diversiloba
Cercocarpus betuloides	R. trilobata
Eriodictyon californica	Sambucus velutina
Fremontia californica	Staphylea bolanderi
Pinus sabiniana	Styrax officinalis

FOREST.—The forest is of the yellow pine type which grades in more mesophytic situations and at higher altitudes into the mixed conifer type. *Pinus ponderosa* is the common and most conspicuous conifer, and associated with it are *Libocedrus decurrens* and *Quercus kelloggii*. The forest area in contact with the chaparral has been both logged and burned, but the stand of yellow pine is extensive and is reproducing. Layer shrubs of *Arctostaphylos mariposa* and *Ceanothus integerrimus* are common. The ground cover is *Chamaebatia foliolosa*.

The area studied in detail is between the San Joaquin River on the north and Kings River on the south, some 25 miles wide and extending from the San Joaquin Valley floor, 300 feet in elevation, to about 4000 feet, the highest elevation at which *P. sabiniana* is found in this area.

Pinus sabiniana, one of the nut pines, is described by JEPSON (5) as 40 to 90 feet high, the trunk in typical trees parting

into a cluster of erect branches which form a broomlike top. It is found on arid foothills, forming a very thin stand on Sierra foothills and coast ranges mostly toward the interior, reaching the coast only in the Santa Lucia Mts. The timber is inferior.

JEPSON (4) states that *P. sabiniana* inhabits the arid foothills of the Sierra Nevada and coast ranges surrounding the oval of the Great Valley of the Sacramento and San Joaquin, save for a curious break of 20–25 miles on either side of the Kaweah River in the southern Sierra Nevada between the Kings River and White River. It is also generally distributed throughout the dry valleys and hills of the north coast ranges as far westward as the borders of the Redwood belt. In the south coast ranges it is common in the Mt. Diablo, Mt. Hamilton, and San Carlos ranges, eastern slope of the Santa Cruz and Santa Lucia Mts., then extends southward to the Mt. Pinon region in Ventura County, and finally to the Sierra de la Liebre south of Tehachapi and Antelope Valley, the southernmost locality.

In ascending the foothills, aside from the stream bank associations dominated by *Populus fremontii*, *Salix nigra* var. *vallicola*, and *Platanus racemosa*, the first trees seen are *Quercus wislizenii*, found on rocky outcrops and along the fissures of the lava caps of the local table mountains, and *Q. douglasii*, frequently in pure stands in level places but tending to occupy the lines of seepage between the rolling frontal foothills. Above the 600-foot level *Pinus sabiniana* appears, and with them forms the dominant tree vegetation of the Upper Sonoran, or area of woodland and chaparral under discussion.

Within its zone of distribution, *Pinus sabiniana* occurs in practically every situation. Not only on the dry hillsides, but approaching close to the banks of perennial streams, with excellent stands on the floodplains of the Kings River and on the banks of the San Joaquin above Friant, the Digger pine is universal.

TOPOGRAPHY AND SOILS

The area selected for intensive study is typical of the southern Sierra Nevada and is situated between the San Joaquin and Kings rivers. Its elevation is from about 600 feet on the low frontal foothills to 4000 feet, the upper limit of the Digger pine in this area.

The general direction of the Sierra Nevada is northwest to southwest, with the foothills having a gradual slope westward and southwestward. The two main rivers debouch from the foothills at an elevation of about 400 feet. They have built up deltas of considerable size at the edge of the foothills and on the floor of the San Joaquin Valley, but a study of their vegetation is not part of this paper. The frontal foothills, which are of the Tertiary and Quaternary periods, have been eroded to a generally rounded appearance with no vegetation except grass and herbaceous material.

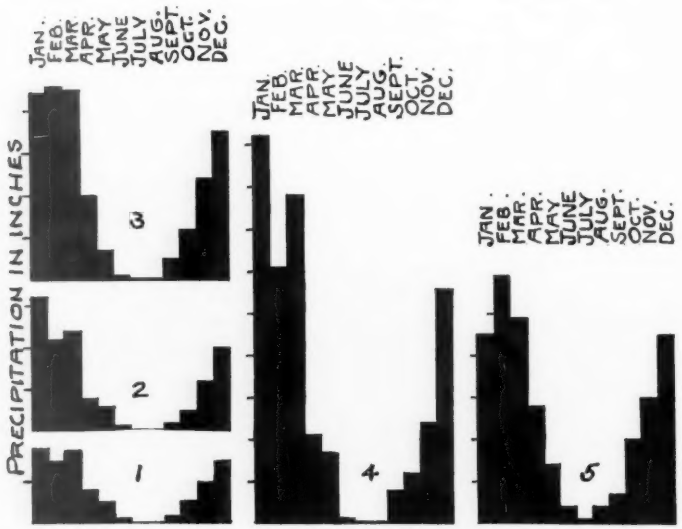
The core of the Sierra Nevada is granite, with a remnant of metamorphosed sedimentaries on the western flank, some remnants on the high crests of the range, and great blocks of sedimentaries abutting against the fault scarps to the east. Aside from relatively small amounts of basalt along the San Joaquin River, therefore, the bulk of the soil-building material is from the weathering of granite rocks. The soils of the brushland and chaparral areas are largely granitic. With increased altitude and precipitation there is an increase in humus, particularly at the upper levels of the distribution of the Digger pine. Local topography with drainage into low spots, coupled with the action of intermittent streams, has in some places caused an accumulation of soils rich in humus; but in general the soils are light and sandy with good drainage.

Climate

The Great Valley of California, whose northern and southern divisions are known as the Sacramento and the San Joaquin valleys respectively, has an average width of about 50 miles. The climate is characterized by its rainless, hot summer and mild winters with light rainfall. There is a slight increase in precipitation from the west to the east side of the valley. With slight modifications, this general characterization applies to the foothill area. The moisture-laden winds from the west are cooled by the Coast Range with resulting precipitation. The eastern slopes of the coast ranges are more xerophytic than the western slopes. The greater height of the Sierra Nevada in relation to the coast ranges enables it to receive enough moisture at higher elevations to support a xero-mesophytic

coniferous forest, while its lower western foothills and eastern slopes are decidedly xerophytic.

In figures 1–5 the distribution of precipitation is shown over a period of years. The woodland and chaparral area extends from an



FIGS. 1–5.—Average monthly distribution of precipitation in inches from January to December at five stations on western slope of Sierra Nevada Mts.: Fig. 1, Fresno, elevation 290 feet, precipitation 9.82 in., 41-year record; grassland. Fig. 2, Friant, elevation 345 feet, precipitation 12.81 in., 24-year record; grassland-oak savanna. Fig. 3, Auberry, elevation 2050 feet, precipitation 23.49 in., 13-year record; Digger pine-oak woodland, chaparral. Fig. 4, Northfork, elevation 3000 feet, precipitation 36.85 in., 15-year record; Digger pine-oak woodland, chaparral. Fig. 5, Big Creek, elevation 4900 feet, precipitation 30.88 in., 13-year record; coniferous forest.

elevation of about 500 feet, just above Friant, to an elevation of approximately 4000 feet at a point above Auberry, depending upon how far fire has driven the yellow pine back and permitted dominance of the chaparral. The precipitation records from Friant, Auberry, and Northfork indicate typical distribution by months in the brushland and chaparral.

Comparing these stations (for all of which records are available for periods of from 13 to 41 years) with Fresno on the floor of the San Joaquin Valley below and Big Creek in the mixed conifer zone above, the distribution of precipitation over the year is comparably uniform, with the maximum in January-March. There is a rapid decrease in April, May, and June; no precipitation or only a small amount at the higher stations during the summer months; and a

TABLE I

AVERAGE MEAN MINIMUM (A) AND AVERAGE MEAN MAXIMUM (B) TEMPERATURES BY MONTHS FOR FIVE STATIONS FROM FLOOR OF GREAT VALLEY TO MIXED CONIFER ZONE

STATION	ELEVATION (FEET)		°F.												NO. OF YEARS
			Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Fresno	300	A	38.0	41.2	44.2	47.3	52.0	59.0	64.6	63.4	57.9	50.6	43.0	38.2	35
		B	54.3	60.9	65.5	73.3	80.9	91.2	99.1	97.6	89.1	78.2	66.1	54.7	35
Clovis (near)	400	A	33.3	36.6	39.3	43.6	48.9	56.1	61.5	59.3	53.9	46.1	38.3	34.9	13
		B	55.2	62.3	67.4	74.2	85.5	95.6	102.8	100.1	93.7	81.2	67.1	55.8	13
Auberry	2050	A	31.6	35.3	38.8	42.3	48.4	57.3	62.9	61.2	53.8	44.7	37.2	32.9	14
		B	54.9	57.8	60.7	67.0	76.9	86.9	95.1	93.5	86.6	74.9	65.0	57.9	14
North-fork	3000	A	29.7	31.8	34.8	38.3	42.4	50.4	57.5	55.4	49.2	41.5	34.8	29.8	21
		B	54.7	56.7	60.7	66.7	75.2	82.5	94.5	92.4	85.3	75.0	65.4	55.7	21
Big Creek	4900	A	31.8	32.8	34.3	38.4	45.9	54.3	61.7	59.9	54.2	46.4	40.2	34.6	14
		B	49.4	50.5	53.5	57.9	66.0	75.6	83.9	82.5	76.2	66.4	58.7	51.2	14

gradual increase in September, October, and November to the maximum again.

Temperature records (table I) are available for a rather long period of years for stations covering this area from the valley floor to well above the zone of woodland and chaparral. The difference between the average mean minima and the average mean maxima during the period of lowest precipitation is rather uniform for the different stations, but the average mean maxima are, as expected, highest at Fresno and Clovis, on the valley floor and the frontal foothills. They are rather uniform for the Auberry and Northfork stations at elevations of 2050 and 3000 feet, and decrease sharply above these points. This seasonal distribution of precipitation, with the maxi-

mum during the winter and a dry summer accompanied by high temperatures, provides the environmental complex for the development of broad sclerophylls as discussed by SCHIMPER (**9**) and COOPER (**3**).

Figures 1–5 indicate that, although maximum precipitation occurs at both Fresno and Big Creek over a period from September to March, the bulk of it is in the four months from December to March with practically none during June, July, and August. Precipitation in the San Joaquin Valley is therefore sufficient to support only grassland, while that of the higher elevations permits the development of coniferous forests, the precipitation of the winter months being sufficient to meet the losses of the dry growing season. The sclerophylls of the intermediate points in the woodland and chaparral are so adapted as to take advantage of any favorable period for growth when moisture and temperature conditions are suitable.

INSTRUMENTAL DATA

In order to obtain data on certain environmental factors, particularly the rate of evaporation in relation to the distribution of *Pinus sabiniana*, atmometers were established at four different points and maintained from April 20 to November 16 during the season of 1929, and from April 5 to October 14 during the season of 1930. Stations were located so as to have one representative of the valley grassland, one representative of the grassland-oak savanna, one representative of the Digger pine-oak woodland, and the highest station at the advancing edge of the chaparral into the yellow pine coniferous forest area.

STATION NO. 1, FRESNO, ELEVATION 300 FEET.—Conditions here are representative of the floor of the San Joaquin Valley. The location was in the college garden and somewhat east of the center of the valley on an east to west line. Although not an ideal location, as neighboring buildings interfered somewhat with the free movement of air currents, yet it seemed the only practicable location from standpoint of accessibility and protection for the atmometers.

STATION NO. 2, FLEMMING RANCH, ELEVATION 450 FEET.—This station was located in the hills at a point in well established oak savanna. *Quercus douglasii* and *Q. wislizenii* are common, but *Pinus*

sabiniana does not occur so far down. The station was some 75 feet from an intermittent stream which usually runs for a few weeks in the spring. Along the stream is a scattered stand of *Populus fremontii* and *Platanus racemosa*. The topography is broken, but a level spot was located, and fenced to prevent depredations by stock. Shrubs and trees immediately around the station are not reproducing, owing to grazing, but on the hills above they are plentiful.

The soil at this station is higher in humus than at any of the other stations except no. 4. An area of metamorphosed sedimentary rock on the flanks of the Sierra Nevada is exposed at this point, which combined with general topography and previous stream action has produced a soil somewhat superior to those of the area as a whole.

STATION NO. 3, HUGHES RANCH, ELEVATION 700 FEET.—*Pinus sabiniana*, as well as *Quercus*, *Ceanothus cuneatus*, and *Aesculus californica*, are well established. Seedlings of *P. sabiniana* and *Ceanothus* are developing within a short distance of the atmometers. In order to find an open spot away from cultivated land and free from shade, it was necessary to locate the atmometers on a slight ridge. The soil of this ridge is a shallow layer of decomposed granite; in fact it was most difficult to get post holes sufficiently deep to put up an adequate fence. The area is representative of the Digger pine-oak woodland.

STATION NO. 4, YELLOW PINE FOREST, ELEVATION 4000 FEET.—This site was selected because of its convenience to the road and its location at the line of contact between the chaparral and the yellow pine forest. Scattered stands of *Pinus sabiniana* occur 25 yards below this station and a few occur above. The general exposure of the ridge upon which the station was located is to the west, and the high altitude of the yellow pine conifer forest cover at this point is undoubtedly due to the encroachment of chaparral following fire. Although *P. ponderosa* is the most common conifer, the typical trees and shrubs of the Transition Zone are present. *Libocedrus decurrens*, *Abies concolor*, and *Quercus kelloggii* occur, while the characteristic *Chamaebatia foliolosa* is the ground cover.

ATMOMETER PROCEDURE

At each station the atmometers were located at ground level with the bulbs about 14 inches above the surface of the soil. They were

situated so as to avoid shade so far as possible, but at station no. 4 shade could not be entirely avoided.

The atmometers were of the standardized sphere type fitted with a Livingston-Thone rainproof valve. During the season of 1929 they were run in pairs, but during the 1930 season black and white spheres were maintained at each station.

Because of the distance from Fresno, it was possible to read the atmometers only fortnightly during the 1929 season, but during the 1930 season they were read weekly throughout most of the period. At each visit the bottles were exchanged for fresh ones containing a known weight of water and the old bottles reweighed to determine the amount of water lost. This proved a more practicable method for field work, in view of the high water loss, than to add water by means of graduates and pipettes. At each visit during 1930 the temperature at the surface of the ground was taken by laying a thermometer on the ground and covering the bulb lightly with trash. An attempt was made to collect soil samples at stations no. 2, 3, and 4 at a depth of 18 inches, but owing to the great variation of the soils of the different stations and the extremely rocky nature of the country, uniformity in sampling was difficult. The soils were collected in tight cans and dried in the laboratory at 105° C. The temperature of the soil at 12 inches' depth was also taken by means of a thermometer, either by lowering it in an auger hole where the ground was hard or by plunging it in soil at that depth when it could be removed by shovel.

Environmental factors

Even to the most casual observer, the marked zonation and grouping of the trees and shrubs is evident as one ascends the foothills from the San Joaquin Valley floor. It is difficult to obtain definite data bearing on this distribution, and particularly to interpret such data, since the problem is complicated by many factors.

The station at Northfork is some 15 miles to the north of the area studied, but conditions of topography and vegetation are identical with the corresponding elevation under discussion. A summary of the available temperature data is given in table I. The outstanding features are the high summer temperature and the wide range between maxima and minima.

The uniformity in the distribution of precipitation has already been noted (figs. 1–5), there being an increase with altitude but the greatest precipitation coming in the same months at higher stations as at the lower stations. It should be pointed out that there is not a direct and uniform increase with altitude but an increase which reaches a maximum and then diminishes with greater increase in altitude.

Considering temperature alone, increase in altitude produces a uniform decrease in the average mean temperature, but as between

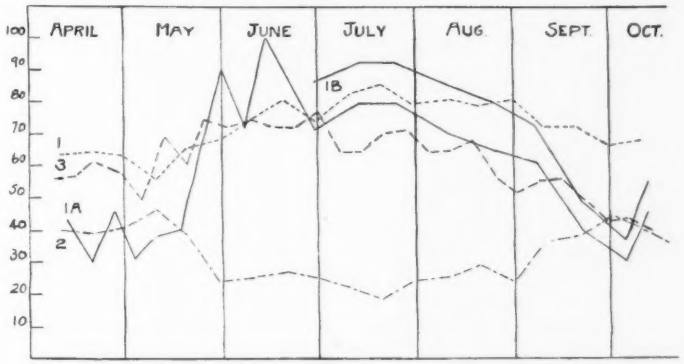


FIG. 6.—(1) Comparison of mean daily temperatures (° F.) at Fresno: evaporation per day in grams from (1A) white spheres and (1B) black spheres; (2) relative humidity; and (3) solar and sky radiation in gram-calories per sq. cm. of horizontal surface; season 1930.

the San Joaquin Valley and the Digger pine-oak woodland, it is a matter of but a few degrees and not sufficient to be a determining factor in distribution. In fact *Pinus sabiniana* has been planted in many places, not only on the valley floor but in the area to the south of Kings River, where it is conspicuously absent in the native vegetation. In all cases it seems to be thriving, with every indication that the life span will be the same as in the area of natural distribution.

In figure 6 the relations of mean daily temperature, relative humidity, solar and sky radiation, and evaporation from white and black atmometer spheres are shown. Although data on humidity and radiation are available only for the valley floor, other data of tempera-

ture and rainfall indicate that comparative conditions exist in the Digger pine-oak woodland of the foothills.

It is to be noted that July, the month of highest temperatures, shows the lowest humidity; while in June occurred a period of high evaporation from the white spheres. June was also the month of highest solar and sky radiation. Black spheres were available from July to the end of the season, and show a parallel increase in water loss as compared with the white spheres.

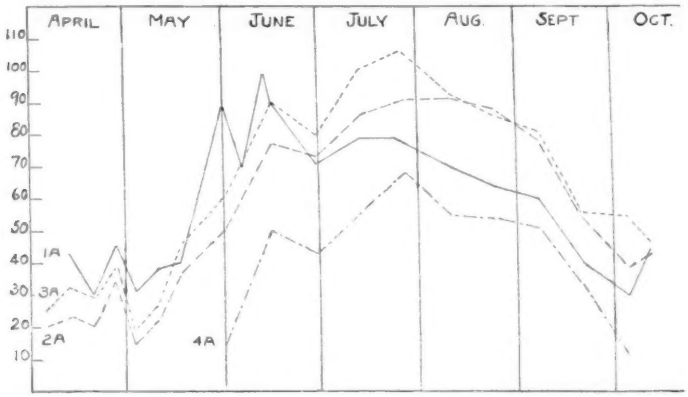


FIG. 7.—Comparison of rate of evaporation per day in grams from white spheres at stations 1, 2, 3, and 4; season 1930.

The relative rate of evaporation from white spheres at all stations as shown in figure 7 indicates that conditions in relation to water loss are more severe on the valley floor in the early summer, but in July conditions in the Digger pine-oak woodland exceed those of the valley floor as well as the oak savanna of the first foothills. This great increase in the evaporating power of the air during July is difficult to explain. In the matter of temperature and precipitation, as all available records show, conditions in this zone are more favorable than in the zones below.

Significant features of the dominant elements of the foothill vegetation, the oaks and the Digger pine, are their tolerance to light and high temperatures and their capacity to develop in spite of the extremely desiccating conditions during the summer months.

Some evidence as to the amount of radiant heat absorption by an evaporating surface is shown in figure 8 by the relative loss from white and black atmometer spheres. Considerable difficulty was experienced in maintaining the black atmometers. They seemed to

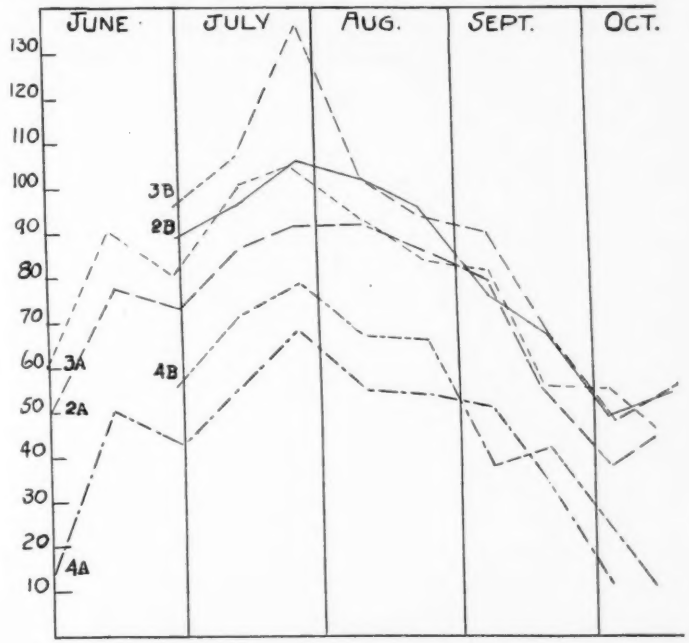


FIG. 8.—Comparison of rate of evaporation per day in grams from (A) white spheres and (B) black spheres at stations 2, 3, and 4; season 1930.

be especially attractive to rodents, who scratched off more or less of the lamp black. All stations showed an increased water loss in the black spheres, especially through June and July, with a marked decrease in the difference between white and black spheres in August and September. The greatest increase in evaporation is at station no. 3 in the Digger pine-oak woodland, and occurred in July.

The influence of ground temperature, soil temperature, and percentage of soil moisture upon the distribution of *Pinus sabiniana* is difficult to interpret. Within its altitudinal range and in the area

under discussion, *P. sabiniana* is found in every sort of situation, from the banks of perennial streams to the very unfavorable conditions of granitic outcrops, basaltic talus slopes, and the slightly weathered tops of basaltic table mountains.

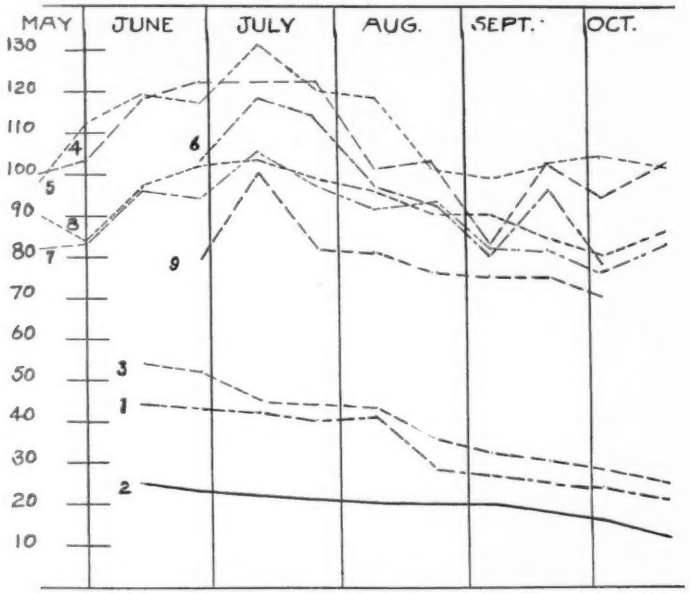


FIG. 9.—Comparison of percentage of soil moisture and surface and soil temperatures (° F.) at stations 2, 3, and 4; season 1930: 1, 2, 3, percentage of soil moisture at stations 2, 3, and 4; 4, 5, 6, surface temperatures at stations 2, 3, and 4; 7, 8, 9, soil temperatures at stations 2, 3, and 4.

The data assembled in figure 9 upon ground and soil temperature and percentage of soil moisture are subject to considerable error. In the matter of ground and soil temperature, it was impossible to take these at exactly the same hour of the day. The difficulty of sampling due to the great variation in topography, rockiness, and extreme dryness and hardness of the soil made uniformity in the matter of soil temperature and the collection of soil samples difficult.

For stations no. 2, 3, and 4 the severest conditions exist in July, with both ground and soil temperature running higher in the Digger

pine-oak woodland than in the oak savanna area. The percentage of soil moisture shows a rather uniform decrease throughout the season, with only marked decrease in August at station no. 2 in the oak savanna.

The generally greater severity of conditions at station no. 3 in the Digger pine-oak woodland, as indicated by the available data, does not seem consistent with the distribution of vegetation. The difference in altitude between stations no. 2, oak savanna, and no. 3, Digger pine-oak woodland, is a matter of about 250 feet and a distance of some 4 miles. *P. sabiniana*, however, is not found in the area of station no. 2, whereas in the area represented by station no. 3 it is a conspicuous and dominant feature of the vegetation. The difference in environmental conditions between stations no. 3 and no. 4 as indicated by these data represents a change toward a much more mesophytic condition, with dominance of a conifer forest just above station no. 4.

Succession

Cooper (3) believes that the chaparral formerly occupied a much greater area on the lower slopes of the Sierra Nevada. From relict evidence in the Sacramento Valley he thinks there is a possibility that it may in times past not only have controlled a much larger portion of the foothill area here discussed, but also may have been dominant in the area now controlled by the valley grassland, or would be controlled by the grassland were it not for the interference of agricultural development.

The vegetation of the area has undoubtedly been greatly modified by a long period of human occupation. There were several large Indian rancherias between the Kings and the San Joaquin rivers throughout the Digger pine-oak woodland at the time of the first white settlements in 1850. This elevation was one of the main centers of Indian habitation and had long been occupied,[2] as evidenced by the numerous rock pot holes which were used for grinding the acorns of *Q. douglasii* and the seeds of *P. sabiniana*. The Indians, according to the early white settlers, made a practice of setting fire to the grass on the foothills with the idea of keeping down the development of brush and maintaining an open parklike woodland.

[2] Verbal information from L. A. Winchell, a pioneer resident.

With the coming of placer miners and the more permanent settlement of the early stockmen this burning practice was continued. Many stockmen today contend for the practice of annual burning

FIGS. 10, 11.—Fig. 10, oak savanna of frontal foothills; *Quercus douglasii* and *Q. wislizenii* with *Avena* spp. in foreground. Fig. 11, Digger pine-oak woodland; *Pinus sabiniana* in foreground with *Q. douglasii* in rear. Dichotomous branching and open broomlike tops are characteristic of former.

to restrict development of brush, as against the Forest Service policy of fire control.

With more permanent settlement of the foothill area, agricultural development has constantly encroached on the native vegetation where topography has permitted it. Clearing for grain fields, orchards, and to a small extent for vineyards has proceeded steadily. This, combined with road building and the great development of the main timber belt above as a recreation area, has materially increased the fire hazard with its resulting effect on vegetation.

The question of succession in the foothill area is a matter of considerable economic importance. The chief possibilities of the area are in its usefulness for grazing, watershed, and recreational purposes. The secondary successions which are resulting from fire become therefore of importance. The Bureau of Forestry (**12**), in its study of cover type in relation to fire control in the forest of northern California, has found that the chaparral and woodlands in relation to other vegetation types rank high in length of fire season, number of incendiary fires, rapidity of burning, acreage burned over, and cost of control. The frequent destruction of the plant cover by fire accelerates erosion and decreases watershed value, while new plant successions interfere with the other possibilities of usefulness of the foothill area.

Types of succession

Owing to the causes just discussed, the native vegetation has been greatly changed by human occupation, and areas of primary succession are limited. Successions in all cases, because of the xeric nature of the habitat, appear to be greatly telescoped with chaparral, shrubs appearing soon after or at the same time as the pioneers. In limited areas primary xeric successions may be seen as follows.

I. Rock faces and crevices.—On the granitic and basaltic rocks, lichens of the crustose type are common. As crevice plants, *Gymnogramme triangularis*, *Pellaea ornithopus*, *Selaginella bigelovii*, *Cotyledon laxa*, *Panicum hirtecaule*, *Nemophila aurita*, and *Phacelia californica* are common.

II. Weathered rocky hill tops-basaltic caps of table mountains.—Weathering here has produced small accumulations of soil and conditions are somewhat more favorable than those in I. Spe-

cies previously listed, with isolated specimens of *Quercus douglasii*, *Q. wislizenii*, and *Pinus sabiniana*, make up the bulk of the vegetation. Where there has been disintegration by weathering a condition approaching the chaparral is attained but with less density of stand. In numerous cases associations of *Rhamnus californica* and *Ceanothus cuneatus* prevail. Basaltic crevices in spring produce regular patterns of flowers when covered with societies of *Baeria chrysostoma*.

III. TALUS AND HILLSIDE SLOPES.—Talus slopes are limited to the sides of the table mountains where the disintegrating lava caps form talus slopes of small area. These terminate on gravelly slopes resulting from gravels and conglomerates underlying the basalt and offer a poor foothold for plants. On south- and west-facing slopes the vegetation is very scanty, but even in these extremely xerophytic conditions *P. sabiniana* occurs. Isolated specimens may be found even to the tops of the tables. With it as individuals or restricted associations are *Q. douglasii*, *Q. wislizenii*, *Rhus diversiloba*, *Lupinus albifrons*, *Senecio douglasii*, *Ceanothus cuneatus*, *Aesculus californica*, and *Sambucus velutina*. On north slopes the vegetation is comparable with the chaparral of higher elevations, with *Q. wislizenii* particularly prominent.

Because of the very nature of the climatic situation, mesic primary successions are extremely limited. The only perennial streams in the foothill area are the two main rivers. Numerous ephemeral streams run for a short time during the winter, but owing to the extreme drought of the last two seasons several of these have been dry. Occasional springs cause some standing water on rock basins during the spring.

SECONDARY SUCCESSION

Secondary succession is initiated by two primary causes: agricultural usage and fire or a combination of both. With the exception of the "table mountains," the topography is rough with low hills rising to a height of 2500–3500 feet. Although there has been considerable decomposition into shallow soils, in many places the granite is exposed in large blocks and patches. Many small valleys have been put under cultivation. In clearing the land fires have been allowed to run to the tops of the hills or to some natural fire break.

In abandoned fields succession may be observed and is much telescoped. In the oak savanna *Quercus douglasii* may be seen invading open grassland, as well as *Ceanothus cuneatus* which develops into dense stands of rather limited area. In especially xerophytic situations *Lotus scoparius* is a pioneer and is succeeded by *Ceanothus*. Much the same succession occurs at somewhat higher elevations, except that with *C. cuneatus* are associated *Arctostaphylos mariposa* (although it does not reach a large size), *Rhus trilobata*, *R. diversiloba*, and *Rhamnus crocea*.

Where the burning has been so severe that practically the whole woodland formation has been destroyed, *Eriodictyon californicum* is the principal pioneer, to be followed by the shrubs of the chaparral.

Where the chaparral itself is destroyed by fire, several of the principal woody elements are able to reproduce themselves promptly by root sprouts. In a permanent quadrat on the Auberry road at an elevation of about 3100 feet, which was burned in July, 1930, crown sprouts of *Quercus wislizenii*, *Aesculus californica*, and *Umbellularia californica* were 15–30 inches high on May 1, 1931. Especially conspicuous features of reproduction in this burn are the large number of seedlings of *Eriodictyon californicum* and the production of *Fremontia californica* from running roots as well as basal sprouts. In numerous cases sprouts from roots were observed as far as 15 feet from the parent plant. They were produced in great numbers and the young shoots were making vigorous growth. In this burn all specimens of *P. sabiniana* and *Arctostaphylos* were killed by the fire of July, 1930. *Quercus*, *Umbellularia*, *Fremontia*, and *Aesculus* are rapidly and successfully renewing themselves by root sprouts, whereas *Arctostaphylos* seedlings were numerous.

The summer of 1931 was extremely severe. The total precipitation was below normal, while for four of the six months from April to September inclusive the mean temperature, according to records at Fresno, exceeded the average mean of the last 34 years.

When this burn was examined in October, 1931, the effect of the desiccating summer upon chaparral plants was found to have been the destruction of practically all the *Arctostaphylos* seedlings, while those forms reproducing by root sprouts, although reduced in numbers and vigor, were maintaining themselves.

The development of seedlings is an interesting feature of this

burn. In the spring of 1931 a large number of herbaceous species, a list of which is attached, invaded the burn with *Montia perfoliata* especially prominent. As the season advanced other herbaceous and

FIGS. 12, 13.—Fig. 12, contact between chaparral and yellow pine forest; atmometer station no. 4. Fig. 13, reproduction in chaparral about 9 months after heavy fire. All *Pinus sabiniana* and *Arctostaphylos mariposa* killed; *Quercus wislizenii* and *Umbellularia californica* rapidly reproducing by root sprouts. Numerous seedlings of *Eriodictyon californicum* in foreground.

woody forms became more prominent, especially the slower-starting woody perennials such as *Eriodictyon californicum*. This species is always a feature of open roadsides and other situations of intense light and heat. To determine the number of seedlings, an accurate count was made of a 5-foot quadrat. There were 21 specimens of *Montia perfoliata*, 76 of *Eriodictyon californicum*, 3 of *Mentzelia dispersa*, and 126 of *Ceanothus divaricatus*. The presence of this last species is interesting as it usually occurs in the upper limits of the chaparral and in this area there are no large stands of mature individuals.

Several local showers had caused a late-season development of grasses and herbs in this burn. The following is a list that could be identified in the permanent quadrat up to June 1, 1931.

Grasses and herbs invading permanent quadrat

Amaranthus graecisans
Anthemis cotula
Arabis holboellii var. arcuta
Avena fatua
A. barbata
Brodiaea capitata
Bromus hordeaceus
B. rubens
Chenopodium carinatum
Cirsium californicum
Collinsia tinctoria
Cryptantha flaccida
Delphinium decorum
Dendromecon rigida
Erodium cicutarium
Eschscholtzia caespitosa
Festuca megalura
Galium nuttallii
Gilia gilioides
Gilia multicaulis
Lotus scoparius
Lupinus benthami
Marrubium vulgare
Matricaria suaveolens
Mentzelia dispersa
Mimulus torreyi
Montia perfoliata
Plagiobothrys nothofulvus
Poa annua
Phacelia californica
Polygonum aviculare
Pterostegia drymarioides
Salvia columbariae
Scutellaria angustifolia
Silene antirrhina
Solanum xantii
Streptanthus diversifolius

Upon other burns in the same vegetative area and under the same conditions of topography where fire occurred 5–10 years previously, the succession has moved forward. The area is covered with a

chaparral growth, 4–8 feet high and considerably tangled. *Quercus wislizenii* composes about 40 per cent of the vegetation, *Eriodictyon* 30 per cent, *Ceanothus cuneatus* 20 per cent, with scattered specimens of other chaparral shrubs making up the remainder. The succession following fire at the edge of the forest area is a chaparral formation which includes a mixture of a wide variety of woody plants, of which *Q. wislizenii*, *Umbellularia californica*, and *Pinus sabiniana* attain tree form; whereas in the oak savanna, and particularly in the Digger pine-oak woodland, the succession form in the pioneer stages tends to be an almost pure stand of *Ceanothus cuneatus*.

FIG. 14.—Reproduction of *Pinus sabiniana* and *Ceanothus cuneatus* after an old fire

The relationship between the chaparral and the line of contact with the yellow pine forest can be seen just above this burned area. The point of contact is approximately 4000 feet in elevation and there are definite indications that the chaparral has pushed back the yellow pine for a noticeable distance. Typical chaparral plants, as *Quercus wislizenii*, *Arctostaphylos mariposa*, and *Pinus sabiniana*, invade for some distance the yellow pine area. In view of the observed susceptibility of *P. sabiniana* to fire, and the youth of the trees, as evidenced by borings, it would seem that this invasion is a succession resulting from fire along the edges of the conifer forest.

On west and south exposures, where the slopes are steep, this chaparral formation tends to persist as a climax, with the arboreal elements, as *Quercus* spp. and *Pinus sabiniana*, tending to crowd out the shrubby specimens to form a climax woodland or sclerophyll forest in contact with a climax yellow pine forest, unless the succession is again checked by fire.

The relation of *Pinus sabiniana* to these successions is significant. It occurs throughout the upper portion of the woodland area here designated as the Digger pine-oak woodland. It is an integral part of the chaparral, and as such is invading the yellow pine forest for short distances as part of the present succession. It does not occur, however, below certain clearly marked levels, usually about 600 feet in elevation, the area below being an oak savanna. This definite zonation seems to be the result of two sets of factors. The first is climatic and the second is involved in its successional relationships. It is a tree particularly adapted to the xerophytic conditions of the foothills; and there is apparently no reason why it should not develop on the first foothills now dominated by the oak savanna, except for the greater hazard of and its particular susceptibility to fire. On its upper limits as part of the chaparral formation it may invade the yellow pine forest. If fire is kept out, it, with the chaparral, will be succeeded by the yellow pine forest.

The peculiar gap in the distribution of *Pinus sabiniana* between the Kings and White rivers can possibly be explained entirely on the basis of fire. In all the burned areas examined this species seems to have suffered most heavily. The vacant area may be accounted for on the supposition that fire runs to natural barriers, such as the rivers, and that it has not as yet succeeded in reestablishing itself. This process is now taking place, for although the Kings River is a difficult barrier, it has again crossed this stream and formed excellent stands on river benches and hill slopes some half mile south of the river.

Summary

1. *Pinus sabiniana*, a pine restricted to California but widely distributed on the interior coast ranges and the lower western slopes of the Sierra Nevada, except for a break between the Kings and White rivers, was studied in its ecological relationships.

2. The woodland formation of which *P. sabiniana* is a part is in contact at its lower limits, at an elevation of 600 feet, with an oak savanna which forms a narrow belt above the grassland of the Great Valley and lower foothills.

3. The area chosen for intensive study is bounded by the Kings and San Joaquin rivers and extends from the floor of the valley to about 4000 feet elevation.

4. Quantitative data were obtained by the use of atmometers at four stations typical of the main vegetative zones, above, below, and within the zones of distribution of this species. Other data were gathered upon precipitation, temperature, ground and soil temperature, and soil water content.

5. In its lower zone of distribution *P. sabiniana* is associated with *Quercus douglasii* and *Q. wislizenii;* in its higher levels it is found with chaparral shrubs and trees such as *Ceanothus cuneatus*, *Arctostaphylos mariposa*, *Fremontia californica*, *Quercus wislizenii*, and *Umbellularia californica*. At its upper limits it grades into hard chaparral, the result of fire or possibly a climax on steep and rocky western and southern slopes.

6. As part of this chaparral it is invading the yellow pine forest on its upper limits but is not moving downward into the oak savanna.

7. Studies of burns, both new and old, show that *P. sabiniana* is easily killed by fire. *Quercus*, *Umbellularia*, *Aesculus*, and *Fremontia*, prominent associates in the chaparral, are reproducing by root sprouts, but *P. sabiniana* must start anew from seed.

8. The data available on environmental factors, as well as a study of succession, indicate that distribution is controlled by its adaptability to the xerophytic conditions of the foothills. It is prevented from intruding into the lower elevations by the greater frequency of fires in the oak savanna, and its upper limits are determined by its relation to the chaparral of which it is a part.

9. Where the chaparral is invading the yellow pine forest as a succession following fire, *P. sabiniana* appears as a part of that succession. If no further fires occur the yellow pine will reestablish itself.

10. The gap in the distribution of *P. sabiniana* between the Kings and the White rivers can possibly be explained entirely upon the relation of this pine to fire, as it has successfully crossed the Kings River, a very formidable barrier, and is slowly advancing southward.

The writer wishes to acknowledge the assistance received from Dr. HENRY C. COWLES in carrying out this work. Thanks are also due to Dr. GEORGE D. FULLER for advice and for the use of instruments, to Mr. W. S. BALLARD for help in maintaining atmometers, and to Mr. C. H. QUIBELL for identifying herbaceous species in the burned areas.

STATE TEACHERS COLLEGE
FRESNO, CALIF.

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CYTOLOGY OF ANTENNARIA[1]

I. NORMAL SPECIES

G. LEDYARD STEBBINS, JR.

(WITH THIRTY-TWO FIGURES)

Introduction

The phenomenon of apomixis in the higher plants has attracted the attention of cytologists ever since its discovery by experimental methods in the middle of the last century (BRAUN 5). The work of JUEL (24) on the European species of *Antennaria*, however, was the first attempt at a comparison of the cytology of closely related sexual and parthenogenetic species; and showed how, in the entirely parthenogenetic *A. alpina*, the reduction divisions in the pistillate flowers are completely omitted. The results of other workers in many other genera, as summed up by ROSENBERG (35), have demonstrated transitions between the presence and the complete absence of the reduction division in apomictic plants, making the further study of the more numerous American species of *Antennaria* seem highly desirable.

That the genus *Antennaria* in North America constitutes a large, polymorphic complex of species was first recognized by GREENE (15, 16) and FERNALD (11). They showed that instead of a single, very variable species, there exist in the northeastern United States eleven more or less closely related species. Moreover, a great number of species from other parts of North America have been described, so that more than 100 species from North America are now represented in the Gray Herbarium.

That many of the species in the eastern United States reproduce parthenogenetically was recognized by GREENE (17), and was demonstrated cytologically for four species by LEAVITT and SPALDING. Their brief preliminary note (28) was never followed by a detailed account of their results.

The present study aims to continue the work of LEAVITT and SPALDING, and to compare the sexual and parthenogenetic species

[1] Contribution from the Laboratory of Plant Morphology, Harvard University.

found in the eastern United States, particularly as regards their reduction division and the development of the gametes in both sexes, in an effort to discover the cause of the widespread parthenogenesis in the genus.

Material and methods

The material of the species studied was collected during the springs of 1928, 1929, and 1930. Nine of the ten species were found in the vicinity of Cambridge, Mass., while *A. solitaria* Rydb. was collected during a trip to Washington, D.C.

The buds were collected from one to two weeks before anthesis; and owing to the great number of heads in an inflorescence, and of flowers in a single head, there was no difficulty in obtaining all the necessary stages of development from the same colony at one time. Herbarium specimens were made from each colony at a later stage, when the plants were mature, and were carefully compared with authentic material in the Gray Herbarium.

The buds were fixed in Carnoy's fluid with the aid of a vacuum pump, and after 24 hours were washed in 95 per cent alcohol. They were then treated with dilute hydrofluoric acid in 70 per cent alcohol for 12 hours, to remove the mineral matter in the hard receptacles and pappus hairs, and imbedded in nitrocellulose according to JEFFREY'S (**23**) method. The staminate heads were imbedded whole, but in the pistillate flowers a larger number of the necessary longitudinal sections was secured by removing them from the receptacles and spreading them out separately on cards. Sections were cut 8–12 μ in thickness for the reduction divisions and embryo sac development, while 15–20 μ was found to be a more satisfactory thickness for the older embryo sacs and young embryos. The sections were stained with Haidenhain's iron-alum haematoxylin, and those of the pistillate material counterstained with eosin.

Observations and drawings were made with a Zeiss microscope, a 1.5 mm. apochromatic objective, and a 10× eyepiece, except in the case of the embryo sacs, where the 3 mm. apochromatic objective with the 10× eyepiece gave sufficient magnification. The drawings were made with the aid of a camera lucida, and many of them were enlarged 1.4 and 2 diameters with the aid of a pantograph. The resulting magnifications are given with the descriptions of the plates.

Observations

The genus *Antennaria* is distinguished from its near relatives in the Compositae by its dioecism. The staminate flowers have only the outer integuments of the achene and a short, abortive style and stigma, while the pistillate flowers have only the rudiments of filaments. The staminate and pistillate plants are further distinguished from each other by a number of secondary sex characters, as described by JUEL (**24**).

MICROSPOROGENESIS IN A. PLANTAGINIFOLIA AND A. NEGLECTA

Since micro- and megasporogenesis in *A. plantaginifolia* and *A. neglecta* are essentially the same, these two species will be described together. *A. plantaginifolia:* no. 253, from Turkey Hill, Arlington, Mass.; no. 260, grassy roadside, South Braintree; no. 486, Canton Junction; *A. neglecta:* nos. 481 and 483, Berlin, Mass.; no. 503, Wilmington, Mass.

The material is not very favorable for the study of the early prophases, and both for this reason and also because it has no connection with the problem of apomixis, the study of these stages was omitted. During diakinesis the chromosomes appear as short rods, most of them paired end to end, although O- and X-shaped pairs are frequent (fig. 1). In later diakinesis fourteen pairs of bivalent chromosomes can frequently be counted with certainty, while unpaired univalents have never been seen in either species at this stage. In the heterotypic metaphase, the bivalent pairs arrange themselves regularly at the equator of the bipolar spindle (fig. 2), and in the polar view the number fourteen can easily be counted (figs. 3, 4). In favorable cell plates, variations in shape and size of cross-sections of the chromosomes can be observed, and it is seen that there are seven sets of two similar pairs of bivalents, the members of each set of two usually being associated or close to each other on the plate. Thus, in figure 2, there are in the upper left-hand corner of the cell plate two small, round bivalents, with two large, oblong ones just below them. Near the lower left-hand corner are two bivalent pairs very closely associated, apparently forming a quadrivalent group, while

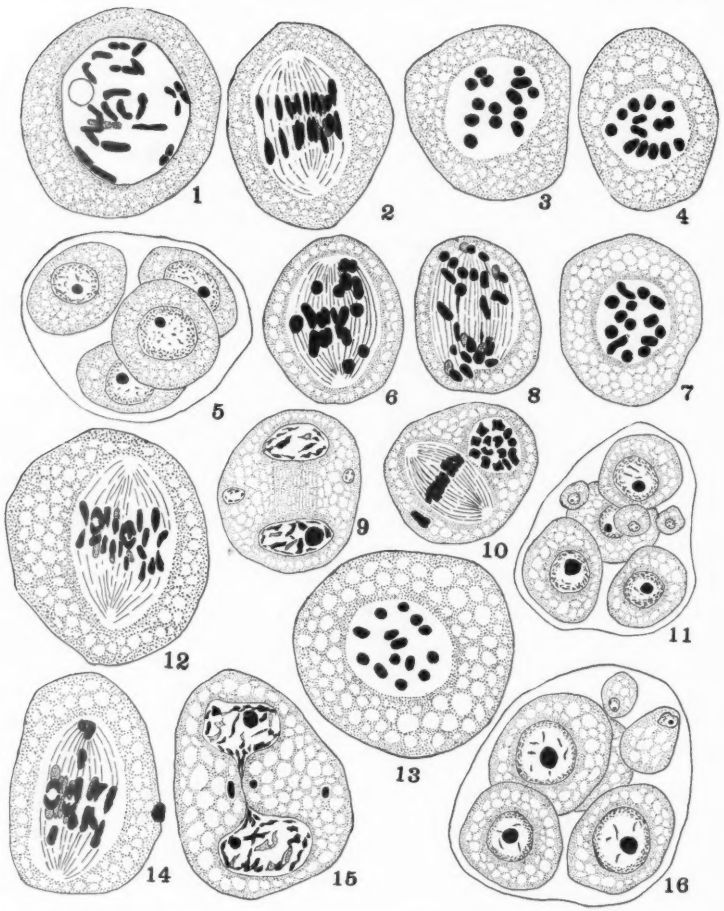
there is a line of four bivalent pairs along the bottom of the cell plate, of which the two pairs at the left are similar in size and perceptibly smaller than the two right-hand ones. Similar arrangements may be seen in figure 3.

A more detailed study of the individuality of the chromosomes in these species will be made in the future, and not until then can the presence or absence of unequal, X-Y pairs of sex chromosomes be definitely established.

The bivalent pairs separate regularly for the early anaphase, although two of them frequently start to separate slightly before the other twelve. They proceed regularly to the poles, and in the heterotypic anaphase fourteen chromosomes can be clearly counted at either pole.

At the end of the anaphase the chromosomes become closely clumped together, and a nuclear membrane forms around them. The interkinesis is long, and the chromosomes lose their identity, although the nucleolus does not generally form. The homoeotypic division is also regular, and in favorable cell plates the fourteen chromosomes can again be counted. The four nuclei formed by this division form a normal tetrad of four microspores, which develop into densely protoplasmic, 2-nucleate pollen grains. All the grains are of the same size, and the pollen is 100 per cent perfect.

Abnormal deviations from this regularity of microsporogenesis were found in one collection of *A. plantaginifolia* (no. 491, from Horn Pond Hill, Winchester, Mass.). This form, although with the leaf, stolon, and inflorescence characters of typical, staminate *A. plantaginifolia*, differed from the typical forms in its pappus hairs with narrower, more sharply serrate tips, thus approaching the shape of the pappus hairs in the pistillate flowers. In this form, lagging chromosomes occasionally occur in the heterotypic division, which are left out of the daughter nuclei at the heterotypic telophase and form small extra nuclei. Thus polysporic "tetrads" are formed. These are about 15 per cent of the total number of tetrads. The aberrant character of the pappus hairs in this form suggests the presence of heterozygosity, which would account for the irregularities observed.



FIGS. 1–16.—Fig. 1, *A. plantaginifolia*, microsporogenesis, diakinesis. Fig. 2, heterotypic metaphase. Fig. 3, same, polar view. Fig. 4, *A. neglecta*, heterotypic metaphase, polar view. Fig. 5, tetrad. Fig. 6, *A. neglecta* × *plantaginifolia*, heterotypic metaphase. Fig. 7, same, polar view. Fig. 8, heterotypic anaphase. Fig. 9, interkinesis. Fig. 10, homoeotypic metaphase. Fig. 11, polysporic heptad. Fig. 12, *A. solitaria*, heterotypic metaphase. Fig. 13, same, polar view. Fig. 14, same, showing irregularities. Fig. 15, interkinesis. Fig. 16, polysporic hexad. ×2400.

Megasporogenesis in A. plantaginifolia and A. neglecta

The following pistillate plants were studied. *A. plantaginifolia:* no. 258, from roadside, South Braintree, Mass.; no. 492, dry wooded hillside, Belmont, Mass.; nos. 506 and 508, grassy hillside, Forest Hills, Boston; no. 749, dry field, Ballston, Virginia. *A. neglecta:* no. 484, dry hillside, Berlin, Mass.; no. 264, grassy knoll, near Braintree, Mass.; no. 517, dry field, Lexington, Mass.

Diakinesis in the megaspore mother cell is similar to that in the microspore mother cell, although the nucleus is in the former somewhat larger, and the chromosomes are more easily counted. The heterotypic division proceeds regularly (figs. 17, 18), and in one or two side views of metaphases and anaphases the haploid number of fourteen chromosomes could be made out. The heterotypic spindle is always oriented along the longitudinal axis of the ovule. At the heterotypic telophase a cell membrane forms, which persists. During interkinesis, the chromosomes lose their identity and a nucleolus forms in each nucleus (fig. 19). The homoeotypic division is regular (fig. 20), and forms a tetrad of megaspore cells with definite cell walls between them (fig. 21).

During both the heterotypic and the homoeotypic divisions there is a definite dense area of cytoplasm or perinuclear zone surrounding the nuclear structures, which is more distinct than that seen in the similar stages of pollen development.

Of the four cells in the megaspore tetrad, the chalazal one soon expands at the expense of the other three, which degenerate. It grows rapidly, and soon pushes through the nucellar cells, which degenerate. Three equational divisions occur, of which the first is shown in figure 28, resulting in a long, narrow, 8-celled embryo sac (fig. 29). This embryo sac expands chiefly at the micropylar end, so that it becomes flask-shaped (fig. 30). The polar nuclei fuse during the early 8-celled stage. The three antipodal nuclei divide repeatedly, forming cell membranes between them, so that at maturity there is an antipodal tissue of 15–20 cells.

Embryo development occurs after fertilization in the usual manner, and in both species pollen tubes were regularly seen in the older material.

Experiments to test the presence or absence of apomixis were performed by the following methods: 1. The styles and stigmas were removed from the unopened buds by cutting across the heads just above the achenes with a sharp razor blade. 2. The pistillate inflorescences were inclosed, before the oldest flowers had opened, in a paraffin paper bag which was removed three or four weeks later.

In both *A. plantaginifolia* and *A. neglecta*, these experiments resulted in complete failure of the achenes to develop, although they were repeated on a number of plants, and untreated heads in the colonies showed a good development of achenes. There is, therefore, no evidence whatever that apomixis exists in either of these two species.

A. NEGLECTA × PLANTAGINIFOLIA.—There are a number of specimens of this hybrid in the Gray Herbarium, while the writer has found that pistillate plants which are evidently intermediate between the two species are not uncommon, although the staminate plants are rarer.

As a guide to the systematic characters of the various hybrid plants studied, a brief summary of the differences between *A. neglecta* and *A. plantaginifolia* is here given.

A. neglecta: Rosette leaves 1.5–5 cm. long by 0.5–1 cm. broad, spatulate, with one prominent midrib; stolons long, procumbent; inflorescence of staminate plants subcapitate, with 2–4 heads; staminate corolla 3.2–4 mm. long; staminate pappus hairs with slightly dilated, crenate tips (fig. 31 *a*); inflorescence of pistillate plants subcapitate, becoming racemose in age, with 2–5 heads; pistillate flowers with corollas 4.5–5.5 mm. long, and with slender, slightly serrate pappus hairs (fig. 31 *b*).

A. plantaginifolia: Rosette leaves 2.5–8 cm. by 0.7–4 cm., ovate-ellipsoid, 3-ribbed, the ribs conspicuous; stolons short, leafy, assurgent; inflorescence of staminate plants subcapitate to corymbose, with 3–12 heads; staminate corollas 2.6–3.3 mm. long; the pappus hairs with much dilated tips and sharply serrate below (fig. 31 *c*); pistillate inflorescence corymbose, heads 5–15; pistillate corollas 3–4 mm. long, the pappus hairs stouter, with long, sharp teeth (fig. 31 *d*).

The hybrids include forms intermediate in these characters.

Hybrid staminate plants

The following staminate plants were studied: no. 478, meadow, Winchester. Leaves 2.5–3.5×0.7–1 cm., spatulate to narrowly elliptic with a single prominent midrib, but more veined than in *A. neglecta;* stolons procumbent; inflorescence subcapitate, with 5–6 heads; corolla 3.3 mm.; pappus hairs intermediate. The chromosomes all pair at diakinesis. In the heterotypic metaphase, most of the cells show fourteen pairs of bivalents at the equatorial plate, but frequently there are two to four unpaired chromosomes, which are scattered near the poles. In the heterotypic anaphase these chromosomes, or sometimes the members of a bivalent pair, lag in the center of the spindle and are left out of the nuclei formed at the heterotypic telophase. They form small extra nuclei at interkinesis, but during the homoeotypic division the nuclear membrane disappears from them, and they show as dark staining masses of chromatin in the cytoplasm.

The homoeotypic division proceeds regularly. At the homoeotypic telophase the four usual nuclei are formed, as well as small extra nuclei, formed around the masses of chromatin in the cytoplasm. This results in polysporic "tetrads." These constitute 15 per cent of the total.

No. 753, dry hillside, Braintree. Stolons not developed; inflorescence subcapitate, with 4–5 heads; pappus hairs intermediate. Here were found 2–4 unpaired chromosomes in 50 per cent of the heterotypic metaphases seen. Polysporic tetrads constitute 26 per cent of the total.

No. 752, dry field, Belmont. Leaves 2.5–3.5×0.7–1 cm., elliptic, pointed, the larger 3-ribbed; stolons becoming assurgent; heads 4–5, in a subcapitate inflorescence; pappus hairs intermediate. In diakinesis the members of one or two pairs of chromosomes are frequently rather loosely associated. In the heterotypic metaphase there are always from 2 to 10 chromosomes lying scattered on the spindle. Frequently two similar chromosomes, obviously the members of a bivalent association which have not paired properly, are seen lying close to each other near a pole of the spindle. Two such chromosomes are seen near the upper pole in figure 6, which shows a case of ex-

treme irregularity for this plant. The haploid number of fourteen can sometimes be counted at the heterotypic metaphase, as in figure 7. The heterotypic anaphase is frequently very irregular, as in figure 8, and there are not uncommonly two to three extra nuclei at interkinesis (fig. 9). The homoeotypic division proceeds regularly, except for the masses of chromatin representing chromosomes left out at the heterotypic telophase (fig. 10). There are sometimes as many as three extra cells in the "tetrads," and polysporic "tetrads" constitute 44 per cent of the total.

No. 490, rocky hillside in scrub, Winchester. Leaves 3–5×0.7–1 cm., elliptic, 3-ribbed; stolons short, assurgent; heads 4–5, in a capitate inflorescence; corolla 2.7 mm.; pappus hairs as in *A. plantaginifolia*. In this form lagging univalents are rare in the heterotypic metaphase and anaphase, and there is only 4 per cent of polysporic "tetrads."

It is thus seen that nos. 478 and 490, which resemble more closely *A. neglecta* and *A. plantaginifolia* respectively, show fewer abnormalities in the reduction division than nos. 752 and 753, which are almost exactly intermediate between the two species.

Hybrid pistillate plants

The following pistillate plants were studied:

No. 514, dry field, Bellevue Hill, Boston. Leaves 2.5–4.5×0.7–1 cm., somewhat elliptic, much veined; stolons becoming assurgent; heads 5–7, in a dense, corymbose inflorescence; corolla 3.8 mm.; pappus hairs with sharp teeth. Mature embryo sacs showed no abnormalities, although there were many degenerate achenes and many flowers in which the stigma was already withered, although the egg had not yet developed. Castration experiments resulted in the apparent parthenocarpic development of a few achenes, but no embryos could be found in any of the castrated or bagged material. The unbagged heads in the same colony showed a fertility of only 0–38 per cent, but some achenes containing embryos were found in all but one of the heads.

No. 495, dry field, golf course, Belmont. Leaves 2–2.5×0.8–1.1 cm., the larger 3-ribbed; stolons becoming assurgent; heads 7; inflorescence corymbose; corolla 4.5 mm.; pappus hairs intermediate. Bag-

ging experiments showed no development of the embryos, while unbagged heads in the same plants produced very few good achenes.

No. 754, dry rocky knoll, Braintree. Leaves 3.5–4.5×0.8–1.1 cm., narrowly elliptic, 1-ribbed; stolons assurgent; heads 3–6, in a dense, corymbose inflorescence; corolla 4.2 mm.; pappus hairs intermediate. Here again bagged inflorescences produced no good achenes, while unbagged heads in the same plant produced a few good, plump achenes.

No. 519, dry field, Lexington. Leaves 2–5×0.9–1.3 cm., narrowly obovate, not strongly veined; stolons mostly procumbent; inflorescence densely corymbose; pappus hairs intermediate. This was the only pistillate form in which the reduction division was observed. At diakinesis there are pairs of bivalent and unpaired univalent chromosomes. Figure 22 shows eleven pairs of bivalents and six univalents. In the heterotypic metaphase there are generally from one to four chromosomes lying scattered at the center of the spindle.

In the heterotypic anaphase there is always some lagging, while figure 25 shows one large and one small extra nucleus at interkinesis. During the homoeotypic division the membranes disappear from the extra nuclei, and they appear as bits of chromatin in the cytoplasm. The homoeotypic division is usually regular. Most of the tetrads have one or two small extra nuclei (fig. 27). The development of the embryo sac is normal up to the young 8-celled stage, the oldest stage observed.

No. 524, clearing in pine woods, Monponsett, Halifax, Mass. Leaves 3–5×0.9–1.5 cm., obovate, rounded; stolons procumbent; inflorescence densely corymbose; heads 8; corolla 3.2 mm.; pappus hairs approaching *A. neglecta*. The youngest stages obtained were tetrads, of which a number contain extra nuclei. The normal development of the embryo sac, illustrated in figures 28–30, occurs in most cases.

One notable exception to this normal development of the embryo sac is illustrated in figure 32. Here a 4-celled embryo sac is degenerating, and another, more densely protoplasmic embryo sac is pushing its way between the chalazal and micropylar ends of the original one, breaking it in two. Only three nuclei were seen in this adventive embryo sac, but its chalazal end was cut off by the knife, and since

the section was not one of a series, this end was lost. Repeated attempts to discover other examples of this phenomenon at different stages, both in this and in other examples of *A. neglecta*×*plantaginifolia*, have failed. It is probable that this adventive embryo sac originated from one of the nucellar cells, although most of them have apparently degenerated, or more likely from one of the cells of the integument. In either case the nuclei of this embryo sac would have the unreduced number of 28 chromosomes.

No castration or bagging experiments were performed on this plant. The negative results of such experiments on all the forms of *A. neglecta*×*plantaginifolia* on which they were performed indicate that this phenomenon is probably of very rare occurrence.

Microsporogenesis in A. solitaria

A. solitaria Rydb., staminate material from Riggs's Mill, Prince George County, Maryland. This species has considerably larger flowering heads than *A. neglecta* or *A. plantaginifolia*, and the anthers and pollen mother cells are correspondingly larger.

At the heterotypic metaphase the chromosomes, although of about the same size as those of *A. neglecta* and *A. plantaginifolia*, are usually more widely separated, and their individuality can more easily be made out. It is seen in figure 12 that they vary considerably in size and shape, and that two pairs of similar size and shape can frequently be discerned. The haploid number of fourteen is easily counted in the polar view of the metaphase cell plate.

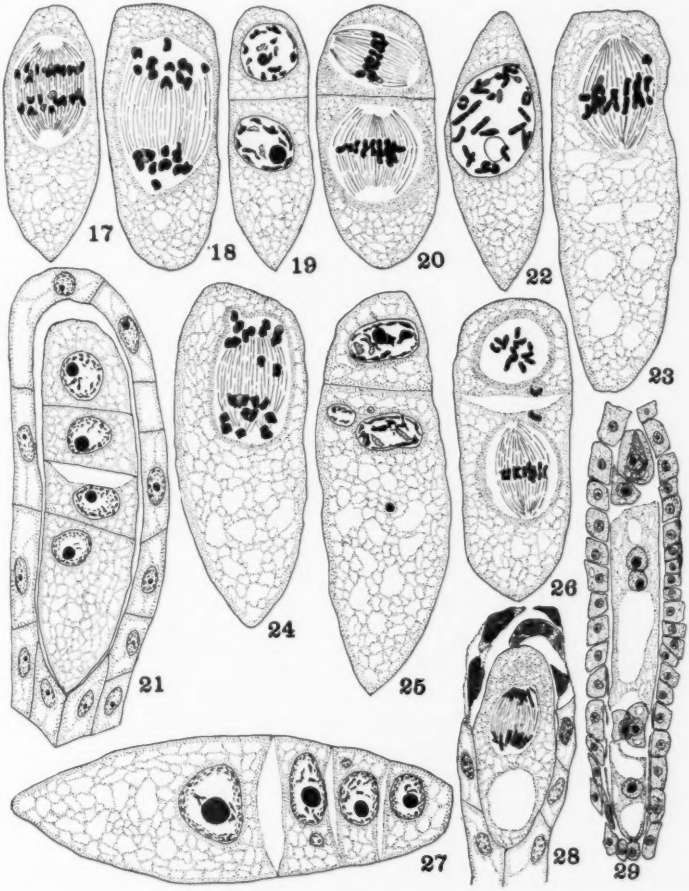
The heterotypic and homoeotypic divisions generally proceed regularly, and a normal tetrad of four microspores is formed. However, unpaired, lagging chromosomes were observed in a very few heterotypic metaphases and anaphases (fig. 14). Figure 15 shows a heterotypic telophase in which a lagging chromosome has been stretched out to form a narrow bridge of chromatin between the two daughter cells. Figure 16 shows a hexad with two microcytes. Such irregularities are rare in *A. solitaria*, and there is only 4 per cent of abnormal tetrads. The pollen is about 98 per cent perfect.

No pistillate material of this species could be obtained.

Discussion

Chromosome number.—The chromosome number of the three sexually reproducing species of *Antennaria* in the northeastern

United States is here established as n = 14. This corresponds with the highest number given by JUEL (24) for the sexual *A. dioica*



FIGS. 17–29.—Fig. 17, *A. plantaginifolia*, megasporogenesis, heterotypic metaphase. Fig. 18, *A. neglecta*, heterotypic metaphase. Fig. 19, *A. plantaginifolia*, interkinesis. Fig. 20, *A. neglecta*, homoeotypic metaphase. Fig. 21, *A. plantaginifolia*, tetrad. Fig. 22, *A. neglecta*×*plantaginifolia*, megasporogenesis, diakinesis. Fig. 23, heterotypic metaphase. Fig. 24, heterotypic anaphase. Fig. 25, interkinesis. Fig. 26, homoeotypic metaphase. Fig. 27, tetrad. Fig. 28, anaphase, first embryo sac division. Fig. 29, young 8-celled embryo sac. Figs. 17–27, ×1800; fig. 28, ×1200; fig. 29, ×600.

Gaertn., which he considered to have between 12 and 14 as the haploid number of chromosomes. From the present study it seems most likely that n = 14 is the correct number for *A. dioica* as well.

Whether this is the fundamental number for the genus is made doubtful by the existence of the groups of similar pairs of bivalents noticed in each species. "Secondary association" was first noted by ISHIKAWA (**22**) in the octoploid *Dahlia variabilis*, where at the heterotypic metaphase the bivalent pairs are grouped more or less definitely into quadrivalent associations. LAWRENCE (**27**) confirmed these observations on *D. variabilis*, and concluded that the species has originated as a hybrid between two tetraploid species. Trivalent groups were noted by BELLING (**2**) in a triploid *Canna*, while BELLING and BLAKESLEE (**3**) noted trivalents and quadrivalents of a similar nature in triploid and tetraploid races of *Datura*, and such groups have been frequently observed since then. LONGLEY (**29**) noted the association of similar bivalent pairs in the tetraploid *Rubus caesius* var. *turkestanicus*, while CRANE and DARLINGTON (**7**) observed complete secondary association in a tetraploid hybrid between the diploid *Rubus rusticanus* var. *inermis* and the tetraploid *R. thyrsiger*. DARLINGTON (**8**) found quadrivalents, as well as secondary association, during the heterotypic metaphase in tetraploid species of *Prunus*, while MEURMAN (**30**) in the 22-ploid *P. laurocerasus* observed sexivalent and octivalent associations of chromosomes.

In *Antennaria neglecta*, *A. plantaginifolia*, and *A. solitaria*, not more than one quadrivalent group of chromosomes is found at the heterotypic metaphase, and this was not clearly distinguished at diakinesis, but the presence of more or less close secondary association at the heterotypic metaphase, and the duplication of chromosome types, make it possible that these species are tetraploids, having originated at some remote period from species with seven as the haploid chromosome number. It is significant that the group of species studied, with their basal rosettes, reduced cauline leaves, and stoloniferous habit are distinctly more specialized and thus apparently more highly evolved than *A. carpathica* (Wg.) Bl. and Fing. and its American relatives, which have no prominent basal rosettes, no prostrate leafy stolons, and possess well developed cauline leaves.

It is likely that some species of this latter group, when examined, will be found to have seven chromosomes as the haploid number.

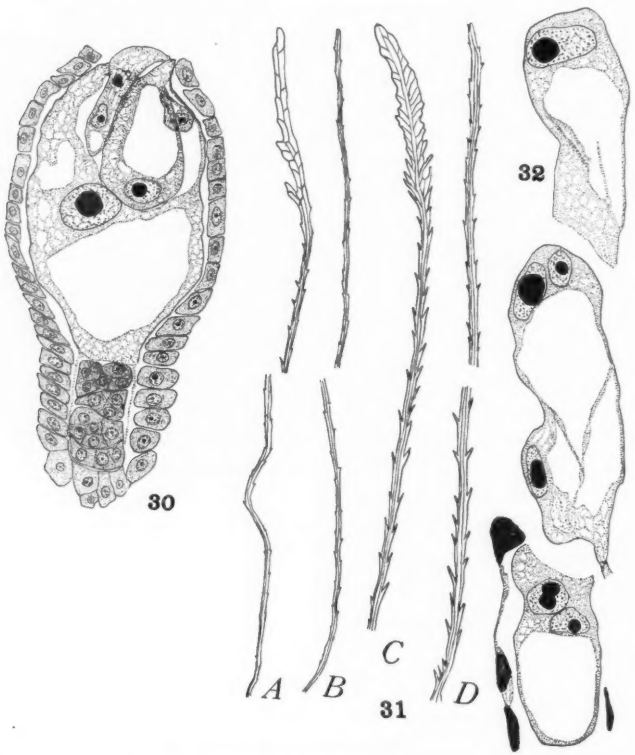
It is notable that *Antennaria* is the only genus yet known of the Compositae in which seven or fourteen is the basic chromosome number (GAISER **13, 14**).

HYBRIDIZATION AND CHROMOSOME IRREGULARITIES.—Since the work of ROSENBERG (**33**) on *Drosera*, the connection of chromosome irregularities with hybridization has been generally recognized, and many workers have since shown the presence of such in known hybrids between species with different chromosome numbers. In hybrids between species having the same number of chromosomes there may be complete homology, and hence complete pairing of the parental chromosomes, with consequent regularity of meiosis, as in hybrids between seven chromosome species of *Fragaria* (ICHIJIMA **21**), *Nicotiana langsdorffii*×*alata* and *N. langsdorffii*×*sanderae* (KOSTOFF **26**); there may be absolute failure of the chromosomes to pair, as in *Digitalis lutea*×*lanata* (HAASE-BESSELL **18**), and *Raphanus sativus*×*Brassica oleracea* (KARPECHENKO **25**); or there may be all stages intermediate between these extremes. The number of paired chromosomes in such forms may vary greatly among different flowers of the same form, and even among different pollen mother cells of the same anther, and is undoubtedly affected considerably by environmental conditions (BORGENSTAM **4,** DE MOL **31**).

In the megaspore mother cells, chromosome irregularities occur less frequently than in the pollen mother cells, but irregularities similar to those in the megasporocytes of *A. neglecta*×*plantaginifolia* occur in *Drosera obovata* (ROSENBERG **33**), the sterile and parthenocarpic varieties of *Musa* (D'ANGREMOND **1**), and the aposporic species of *Hieracium* (ROSENBERG **32, 34**), where polysporic tetrads closely resembling those of *A. neglecta*×*plantaginifolia* are described.

That failure of non-homologous chromosomes to pair is the chief cause of the irregularities in *A. neglecta*×*plantaginifolia* nevertheless seems evident, since, as pointed out, the most nearly intermediate forms, which should have an equal number of *neglecta* and *plantaginifolia* chromosomes, show less pairing of bivalents than those more closely resembling one or the other parent, in which, if chromosomes are the bearers of hereditary characters, there should (probably as a

result of back crossing) be a preponderance of homologous *neglecta* or *plantaginifolia* chromosomes.



FIGS. 30–32.—Fig. 30, *A. neglecta*×*plantaginifolia*, mature embryo sac; ×600. Fig. 31, pappus hairs: *A*, *A. neglecta*, staminate flower; *B*, pistillate; *C*, *A. plantaginifolia*, staminate flower; *D*, pistillate; ×30. Fig. 32, *A. neglecta*×*plantaginifolia*, aposporic embryo sac. ×1200.

APOSPORY.—The aposporic embryo sac in *A. neglecta*×*plantaginifolia* no. 524, although a single case, yet merits attention. Aposporic embryo sacs of this type were first described by ROSENBERG (**32, 34**) in *Hieracium*, and have since been found in *Artemisia nitida* (CHIARUGI **6**), *Ochna multiflora* (FRANCINI **12**), and *Oxyria digyna* (EDMAN **9**).

The existence of this phenomenon in a presumably hybrid form,

in whose probable parents no such phenomenon was observed, leads to the belief that the apospory resulted from hybridization, thus lending support to the theory of ERNST (**10**) and WINGE (**37**). Other cases of apomixis resulting from hybridization are, however, rare. STRASBURGER (**36**) found signs of "apogamy" in *Alchemilla gemmia*, by some investigators considered of hybrid origin, and complete sterility and "apogamy" in *A. trullata*, also of supposed hybrid origin. HARRISON (**19**) and HARRISON and PEACOCK (**20**) found four parthenogenetic females in the progeny of the Lepidoptera *Tephrosia bistortata*×*T. crepuscularia*. In a subsequent paper evidence will be produced to indicate that the regularly apomictic species of *Antennaria* may also be of hybrid origin.

Summary

1. *Antennaria neglecta* Greene and *A. plantaginifolia* (L.) Richards. both have a haploid chromosome number of fourteen, and regular meiosis in both the micro- and megasporocytes.

2. In both species there are seven groups of two similar bivalent pairs.

3. Experiments demonstrated the complete absence of apomixis in both species.

4. In the hybrid *A. neglecta*×*plantaginifolia*, irregularities of meiosis occur, these being most abundant in forms most nearly intermediate between the two species.

5. In one form of the hybrid a presumably aposporic embryo sac was observed.

6. In *A. solitaria* the haploid chromosome number is fourteen, and the meiosis in the pollen mother cells is mostly regular.

7. The basic chromosome number in *Antennaria* is probably n = 7.

8. The chromosome irregularities in *A. neglecta*×*plantaginifolia* are due chiefly to non-homology of chromosomes, but are probably affected by other causes.

9. The aposporic embryo sac in *A. neglecta*×*plantaginifolia* is probably the result of hybridization.

COLGATE UNIVERSITY
HAMILTON, NEW YORK

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VASCULAR SYSTEM OF YOUNG PLANTS OF MEDICAGO SATIVA

CLARA WOLFANGER WINTER

(WITH THIRTY-FOUR FIGURES)

Introduction

The vascular system of a spermatophytic plant, taken in its entirety, forms a conducting channel through root and shoot, being continued into leaves and branches of the shoot by means of prolongations of the main stelar axis of the stem.

The most critical point in the establishment of the continuity of the vascular axis lies in the region of "transition" between the primary root and the primary shoot. The change from the stelar structure of the root, consisting of exarch strands of xylem with phloem on alternate radii, to the stelar structure of the stem, consisting of endarch xylem collaterally arranged with the phloem, presents a situation which has been variously interpreted by anatomists. The trend toward phylogenetic interpretations of anatomical structures has been a stimulus for an increased investigation of this region.

Of the various theories as to the method of transition, a commonly accepted one is that which prescribes definite types of forking, rotation, and fusion of continuous stem-to-root bundles. Three of these types were proposed by VAN TIEGHEM (**6**) in 1871, and a fourth by SARGANT (**4**) in 1900. The theory presupposes a primitive homogeneity of the primary vascular structure which is not consonant with the situation as observed in *Medicago sativa* L. In this plant, the stelar structures of the organs of the shoot, that is, stem, branches, and leaves, are homogeneous to one another and to the secondary, not the primary, vascular tissues of the root.

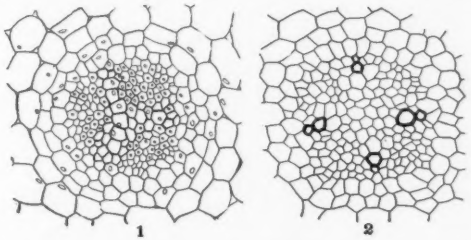
CHAUVEAUD'S (**1**) monumental work on the vascular system presents a recapitulative interpretation of the phenomena observed in the transition region which singularly explains the apparent complications. It is found that seeming anomalies in the primary vascular structure of the seedling of *M. sativa* can be rather nicely explained by CHAUVEAUD'S theory.

The bases for this conclusion are observations made by means of serial sections of numerous seedlings and young plants; by means of portions of shoot and root and of entire seedlings cleared in cedar oil or balsam; and by a study of vascular tissues freed from softer imbedding tissues by maceration methods. This paper reports the details of the structure of the vascular system thus studied.

Vascular system

Root

The first evidences of the vascular system appear about 0.25 mm. above the tip of the root. Three, or more rarely four, xylem strands



FIGS. 1, 2.—Transverse sections through root hair region; fig. 1, 2-day-old seedling showing triarchy; fig. 2, 3-day-old seedling showing tetrarchy. ×230.

with alternating phloem groups are differentiated (figs. 1, 2). Lignification and maturation progress centripetally, and definite strong rays are produced (fig. 3). Where tetrarchy is established in the piliferous zone, the lateral roots may arise from any one of the four rays. More frequently, however, triarchy, with only three longitudinal rows of lateral roots (fig. 4) is evident up to the "collet" region. At this point triarchy, when present, invariably gives way to tetrarchy. In some cases a strong (fig. 9) and in others an abortive (fig. 5) fourth ray is developed. In either case, four very definite, alternating phloem groups are formed.

Instability in the number of root rays, according to COMPTON (2), is typical in many tribes of the Leguminosae, the number varying from two to four. It is noteworthy that in *M. sativa*, tetrarchy, although sometimes abortive with regard to the xylem ray, invariably becomes established as the final stage in primary root develop-

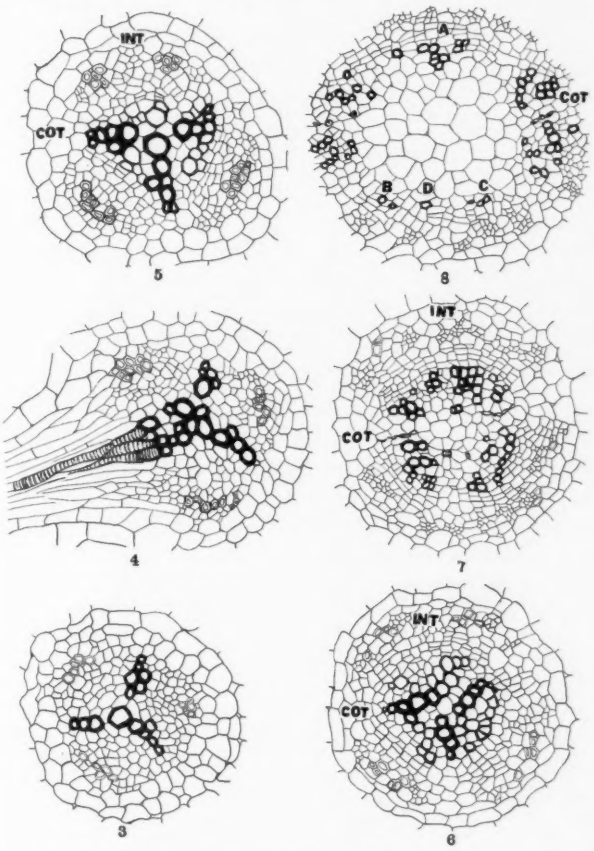
ment. When triarchy is present in the lower portion of the root, the three rays rotate their positions to allow for the development of a fourth. In the triarch stage the angles between the rays are practically equal and the phloem lies on alternate radii between them (fig. 3). Two of the rays, later identified as those in the cotyledonary plane and commonly called the polar xylems, gradually increase the angle between them until they come to lie end-to-end at an angle of 180° (fig. 5 *COT*). The third ray, now lying at right angles to them in the intercotyledonary plane (*INT*), becomes lateral. The fourth ray, when formed, also develops in the intercotyledonary plane, opposite the third ray. A fourth phloem group meanwhile appears, the original three having changed positions simultaneously with the xylem groups so as to retain their alternate positions (fig. 5). Tetrarchy persists through a considerable portion of the root. Seedlings just unfolded show tetrarchy for a distance of 0.75 mm. or more. Plants at the age of two weeks show this condition for a distance of 5 mm. Additional phloem groups, two flanking each of the original ones, arise in this region (fig. 6). They are the first evidences of the second or intermediate phase of CHAUVEAUD (1). When the original phloem groups are not distinctly differentiated, it appears at this stage as if there were eight groups, a pair lying at the outer edge of each xylem ray (fig. 9).

FORMATION OF COTYLEDONARY TRACES

The drawings represent selections from serial transverse sections of a 6-day-old plant. The sections progress upward from the point where the first evidences of tendencies to modify the tetrarch structure appear. The early dominance of secondary tissue in this region is shown by a few sections from a series of an 8-day-old plant (figs. 5–8). The cotyledonary axis (*COT*) and the intercotyledonary axis (*INT*) are shown throughout both series.

The gradual change in position of the vascular elements in the hypocotyl and the ultimate formation of the cotyledonary traces are shown by figures 9 to 18. The entire primary vascular system of the root is continuous with the vascular system of the cotyledons, each cotyledonary trace being made up of a polar and a part of each lateral xylem group, in the following manner. There is a gradual

spreading of the vascular elements toward the periphery of the stele. The outward movement is more pronounced along the cotyledonary axis of the hypocotyl. In transverse section, therefore, the



FIGS. 3–8.—Transverse sections selected from a series through root and hypocotyl of 8-day-old plant: *COT*, cotyledonary plane; *INT*, intercotyledonary plane. Fig. 3, triarch stage with three phloem groups. Fig. 4, formation of lateral root. Fig. 5, rotation of three xylem rays and formation of abortive fourth ray; appearance of fourth phloem group, cambium, and secondary tissue. Figs. 6–8, formation of cotyledonary traces; *A*, *B*, *C*, *D*, secondary vessels which are continuous with plumular traces to first leaves.

central cylinder assumes more and more the appearance of an ellipse with the polar rays on the long axis (figs. 13–15).

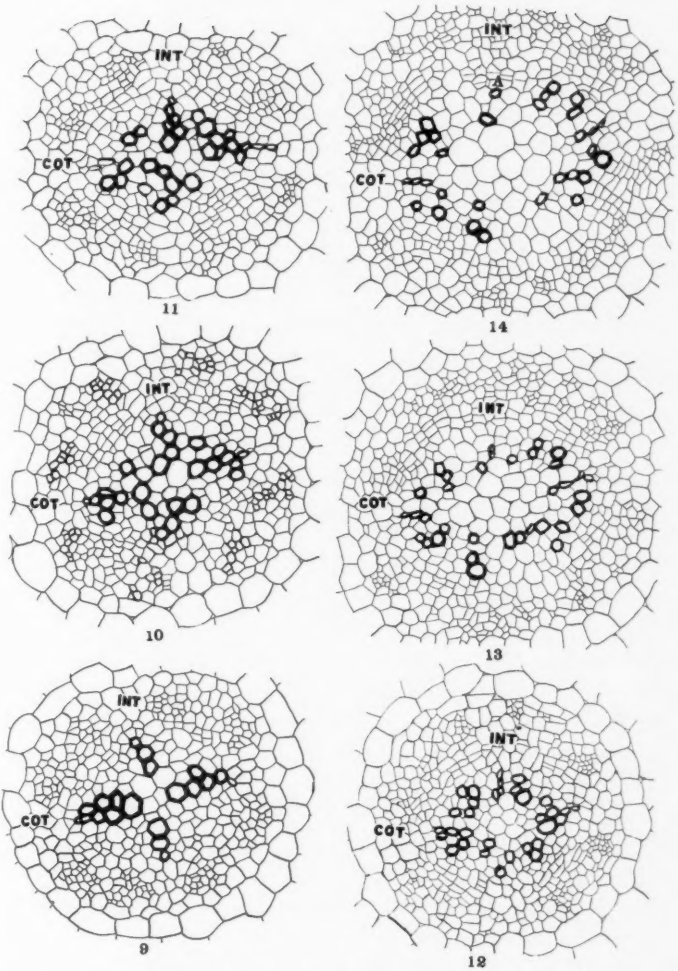
The metaxylem elements of the rays become somewhat separated from the protoxylem elements. Together with secondary vessels early arising from the cambium (figs. 6, 7), they form loose groups, one on either side of each protoxylem strand (figs. 10–12). External to each of the groups thus formed lies a phloem group, one of the eight previously mentioned. The polar protoxylem elements retain their identity and position in the cotyledonary plane throughout, although rapid elongation stretches and distorts them. This is especially evident as they near the cotyledonary node (figs. 13–15). The lateral protoxylem vessels retain their identity in the intercotyledonary plane (*INT*) for some distance, but eventually become disorganized and disappear (figs. 12, 13).

The groups of vascular tissue now constituting each of the intercotyledonary xylems continue to separate, curving toward the cotyledonary xylems so that, eventually, some vessels from each of the lateral xylems reinforce the xylem groups which lie on the right and on the left of the polar protoxylems (figs. 14, 15). The lateral phloem groups change position simultaneously with the xylem, and come to lie, together with the polar phloem, collateral to the two xylem groups on either side of the polar protoxylem (figs. 15, 16, 18).

The resulting structure constitutes the double bundle of THOMAS (5) or, more aptly, the triad bundle of COMPTON (3), being a protoxylem strand, flanked obliquely on either side by a collateral bundle. It is most distinctly seen at the base of the cotyledon where it definitely becomes the cotyledonary trace (figs. 16, 18). Thus the entire primary vascular system of the seedling, as well as some secondary tissues in older plants, is continuous with the vascular system of the cotyledons, each cotyledon receiving a triad (figs. 16, 18).

COTYLEDON

As the triad structure continues into the petiole of the cotyledon, the median protoxylem becomes disorganized and gradually disappears. A branch separates laterally from each bundle of the triad just before the disappearance of the median protoxylem, and the remaining portions of the double bundle fuse to form the collateral



FIGS. 9–14.—Selections from a series of transverse sections of a 6-day-old plant: *COT*, cotyledonary plane; *INT*, intercotyledonary plane; fig. 9, tetrarch stage, eight phloem groups; figs. 10–12, bifurcation of metaxylem; beginning of pith; fig. 13, disappearance of intercotyledonary protoxylem; elongation of cotyledonary axis; movement of intercotyledonary xylem toward cotyledonary poles; fig. 14, triad structure becoming obvious; appearance of *A*, which is continuous with plumular foliar trace. ×250.

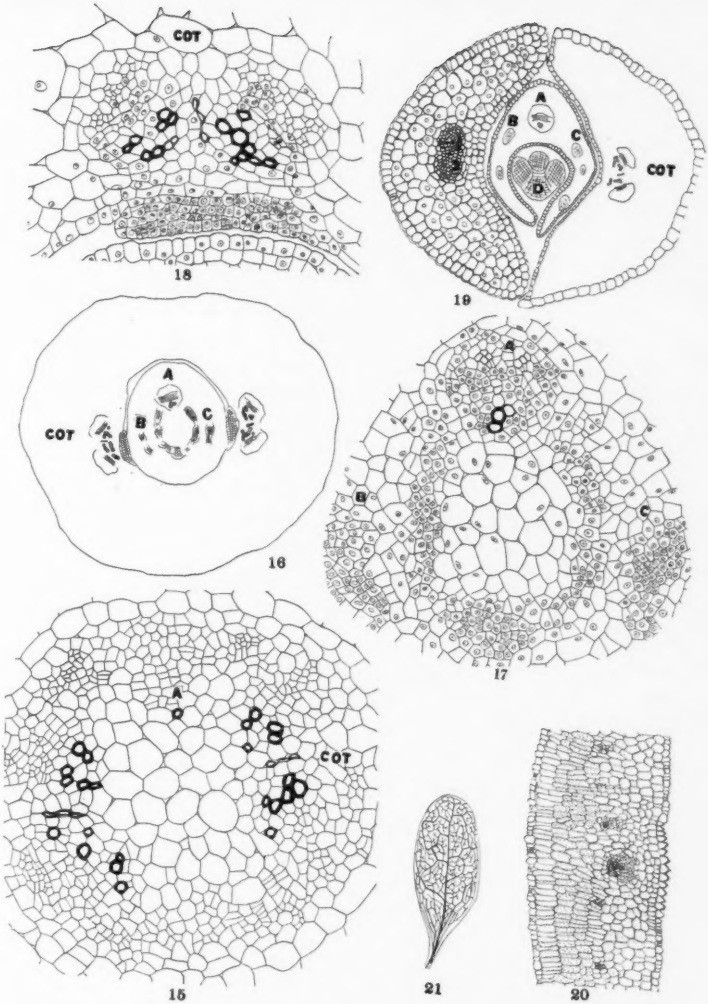
midrib strand of the cotyledon (fig. 20). Numerous other branch veinlets arise, forming an intricate system of venation (fig. 21). Thus, in the cotyledon lamina, the superposed phase of CHAUVEAUD (1) is the only phase to develop, recapitulation being accelerated and the earlier phases being suppressed.

PLUMULAR DEVELOPMENT

There is a decided pause between the development of the primitive vascular structure of the seedling and the superposed vascular structure of the plumule. The vessels arising from the procambium in the plumule are continuous with elements produced by the cambium in the hypocotyl and root. The first vessels in the hypocotyl which are continuous with vessels of the plumule are seen in the intercotyledonary plane at the point where the elements of the lateral xylem rays turn from their position to join the polar rays (figs. 14, 15 *A*). Thus these first vessels are superposed upon the seedling structure in the region of either the third or fourth xylem ray. In the latter case, when the fourth ray is largely an abortive one, they are especially evident in the intercotyledonary plane because of the absence of the elements of the fourth ray (figs. 7, 8 *A*). They are made up of collaterally placed xylem and phloem elements produced by the cambium in this region and, continuing upward, become the median trace to the first plumular leaf (figs. 16 *A*, 17 *A*, 19 *A*).

Three traces supply the first leaf, the median trace (*A*) already mentioned and two lateral traces (*B*, *C*). The two lateral traces arise on the side of the central cylinder opposite the median trace, one on either side of the intercotyledonary axis (figs. 8, 16, 17, 19 *B* and *C*). In a 6-day-old plant they appear as two groups of procambium (figs. 16, 17, 19). Before joining the median trace in the petiole of the first leaf, they give rise to numerous branches which supply the stipules (fig. 19). The median trace to the second leaf lies opposite the median trace of the first leaf on the other side of the vascular cylinder (figs. 8, 19 *D*).

The vascular cylinder of the plumule of the seedling consists of only meristematic tissue or at the most of a few vessels and procambial strands. The foliar traces become separated from the vascular cylinder less than 0.5 mm. above the cotyledonary node (fig. 19).



FIGS. 15–21.—Figs. 15–19, transverse sections of a 6-day-old plant continued from fig. 14: fig. 15, cotyledonary node before separation of cotyledons from main axis of plant; fig. 16, diagram of cotyledonary node: *COT*, cotyledon; *A*, *B*, *C*, traces to first leaf, bud in axil of cotyledon; figs. 17, 18, detail of fig. 16: fig. 17, plumule; fig. 18, triad bundle of cotyledon; fig. 19, cotyledons separated from plumular bud which lies between them; fig. 20, cross-section of cotyledon lamina; fig. 21, cotyledon. Figs. 15, 17, 18, ×250; figs. 16, 19, 20, ×50; fig. 21, ×3.

It is evident that the plumule of the seedling is merely a bud between the two cotyledons.

In older plants evidences of the priority of development of traces to the first and second leaves are lost because of the strong development of secondary vascular tissues. These traces become merely bundles separating from the more or less continuous vascular cylinder much in the manner of subsequent leaf traces. All the primary vascular vessels become imbedded in secondary tissue.

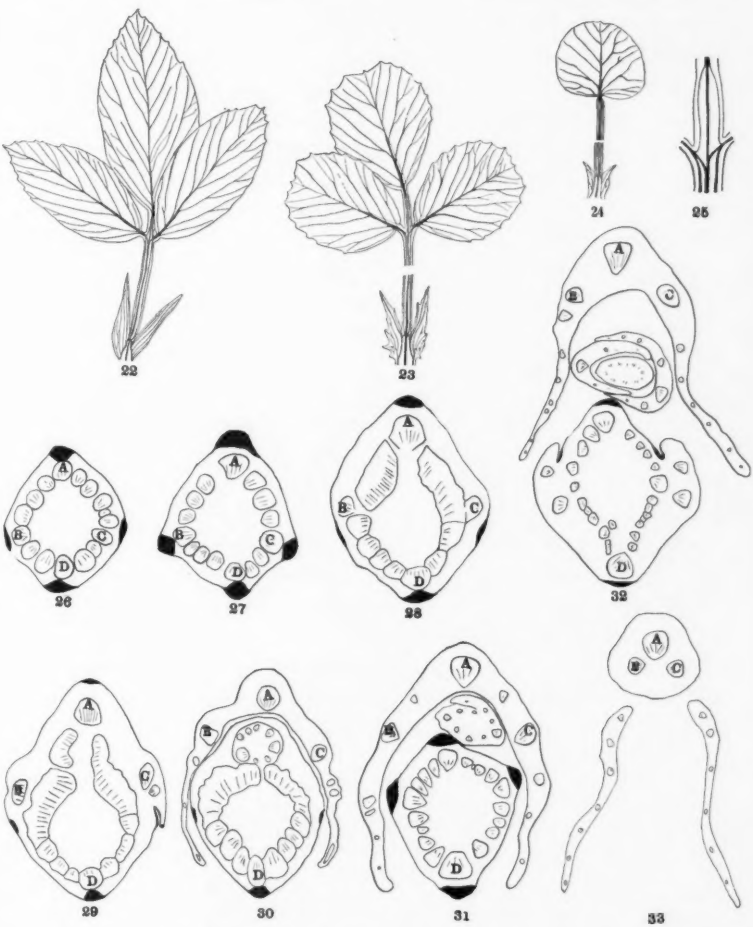
Mature shoot

The first leaf is unifoliolate (fig. 24) and all subsequent leaves are trifoliolate (figs. 22, 23). The stipules at the bases of the leaves are prominent and leaflike. The internode between the first and second leaf is very short in the bud, but elongates greatly in the maturing plant. A bud lies in the axil of each cotyledon (figs. 16, 18) and in the axil of each leaf. These buds, together with the plumule, initiate the stems which arise from the crown of the plant.

Three vascular bundles constitute the foliar traces in both unifoliolate and trifoliolate leaves. The two lateral traces furnish veinlets for the two stipules (figs. 22–24). The median trace enters the petiole directly without branching. The three traces anastomose at the point where the stipules become distinct from the petiole but separate again and are seen in the petiole as three distinct bundles (figs. 22–24).

The unifoliolate leaf is similar to the trifoliolate leaves in vascular structure of stipule and petiole up to the point where the bundles enter the leaflets. At this point, in the unifoliolate leaf, the three bundles from the petiole fuse and enter the one leaflet as a single bundle (fig. 24). In the case of the trifoliolate leaf, the terminal leaflet receives the median bundle and each of the two lower leaflets receive a lateral bundle. However, just as the two lateral bundles turn to enter the two lower leaflets, each is reinforced by a strand from the median bundle and at the same time each sends a strand to join the median bundle. The actual junction of the latter does not occur, however, until the median bundle enters the terminal leaflet (fig. 25). The leaflets are pinnately net-veined (figs. 22–24). Details of venation, showing branching to vein islets, are illustrated by Wilson (7).

The change in the shape of the leaf blade from the cordate outline of the first formed leaflets to the obovate outline of the mature



FIGS. 22–33.—Fig. 22, mature leaf with stipules attached; fig. 23, young leaf; fig. 24, unifoliolate leaf; fig. 25, vascular system of petiole as it passes into the three leaflets; figs. 26–33, successive diagrams showing departure of leaf and bud traces: *A* and *D*, median foliar traces; *B* and *C*, lateral foliar traces.

leaflets is a feature which *M. sativa* shares in common with many of the Trifolieae, according to COMPTON (**2**) (figs. 22, 23).

The origin of the vascular strands which form the traces to the leaves and branches is represented by successive transverse sections of the stem (figs. 26–33) and by a schematic diagram of four nodes of the stem (fig. 34). In figure 34 the internodes are shortened and the width of the trace bundles much reduced.

The phyllotaxy being alternate, the three leaf traces alternate from node to node. The bundles constituting the median leaf traces (*A* and *D*) are broad (figs. 26–32). They originate in two series, being formed on opposite sides of the stem by the union of two strands, one from each of a pair of neighboring stem bundles (fig. 34). These stem bundles retain their identity from node to node, giving rise to successive trace bundles at every other node. Thus the bundles which form the median leaf traces on either side of the stem become identities in the vascular cylinder two nodes below their point of departure (fig. 34). Departure is somewhat abrupt, the stem becoming considerably enlarged on the trace side.

Only one stem bundle gives rise to each of the two series of lateral leaf traces. These stem bundles lie on the vascular cylinder at right angles to the axis on which the median bundles arise, or, in other words, one-fourth of the way around the central cylinder from either median bundle. A strand separates from each of the stem bundles at each node and becomes a lateral leaf trace at the node above (fig. 34 *B* and *C*). These strands (*B* and *C*) therefore become definite bundles in the vascular ring at only one node below their point of departure. They alternate from side to side at successive nodes (fig. 34).

At each node, traces to the branch bud which lies in the axil of the leaf arise just above the departed median leaf trace from the identical pair of vascular stem bundles which form the median leaf traces (fig. 34).

Figure 26 represents the stem cross-section in the region of the internode just below a node. Bundle *A* is the median foliar trace bundle. Bundles *B* and *C* are the lateral foliar trace bundles. In figure 27, the traces have separated slightly from the stem cylinder. As separation continues (figs. 28–32), the portion of the stem in

which these bundles lie increases in size and finally becomes distinct from the stem. The three foliar gaps caused by the departure of the three traces are closed above. Figure 33 represents a transverse section through the petiole and stipules at a higher level, where *A*, *B*, and *C* are petiolar bundles. The vascular strands shown in the stipules are merely smaller branches arisen from the lateral trace bundles (*B* and *C*). Just above the point where the median leaf traces separate from the stem axis, the branch traces also leave the vascular ring (figs. 28, 29). The gap formed by their departure is promptly closed. They form the miniature vascular ring of the bud (fig. 30). The young stem initiates petiolar and stipular formation in the exact manner of the parent stem (figs. 31, 32).

At the time the bundles *A*, *B*, and *C* have become distinct from the main axis, the alternating median trace bundle (*D*) becomes evident opposite bundle *A*. Two lateral bundles, corresponding to *B* and *C*, also separate from the main axial cylinder. The process of stipular, petiolar, and branch formation proceeds as indicated but on the opposite side of the stem (figs. 30–32). WILSON (7) states, "the corners of the somewhat four-angled stem show a multiplication of the cell layers under the epidermis seemingly serving for greater strength." These

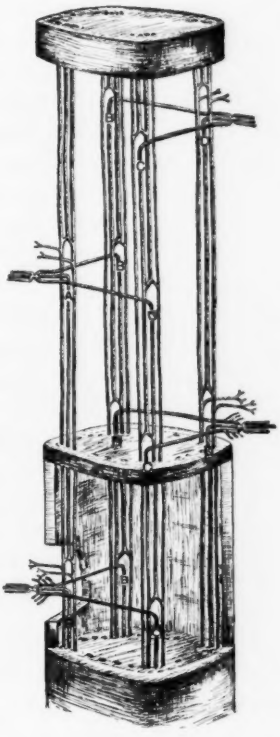


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FIG. 34.*—Schematic diagram of vascular system of stem showing four nodes: internodes shortened and width of vascular bundles much reduced; *A* and *D* are median leaf trace bundles, *C* and *B* are lateral trace bundles; the three traces anastomose before entering the petiole; the lateral traces give rise to branches which supply the stipules; branch traces depart above leaf traces.

* Drawn for this study by JOHN MACK WINTER.

cells are collenchymatous. They occur at the corners opposite each trace bundle (fig. 26).

More or less anastomosing occurs at the node on the side of the departing traces. Between nodes there are no anastomoses. The pattern of the vascular system is seen, therefore, to be comparatively simple as it passes through the stem.

Discussion

In a study of *Medicago sativa* it is found that the primary vascular system of the root, hypocotyl, and cotyledons forms a complete circulatory system of its own, independent of subsequent plumular development. This situation is in agreement with COMPTON'S (2) generalization for the vast majority of epigeal seedlings. The primary system recapitulates the phases of evolutionary development of vascular tissues as described by CHAUVEAUD (1) in his phanerogamic cycle, passing from root to cotyledon through the exarch, alternate arrangement to the intermediate phase, and finally to the endarch, collateral, or superposed condition.

The appearance of the vascular elements in the hypocotyl region can best be interpreted by the recapitulation theory of CHAUVEAUD. The radial arrangement of vascular elements, or the alternate phase, persists throughout the region, being especially evident in the cotyledonary plane. Metaxylem elements, added more or less tangentially on either side of the protoxylem, illustrate the intermediate phase of development. Almost simultaneously with this metaxylem formation, collateral elements arise, further augmenting the vascular system and illustrating the superposed phase. In older plants the primary tissues, constituting the first two phases, become disorganized, and collateral, secondary tissues become the dominant ones. The capacity for the production of the more primitive phases seems to be transient in this region.

Interpreted by CHAUVEAUD'S theory, the singular structure constituting the cotyledonary trace ceases to be an anomalism. The median protoxylem strand is a recapitulation of the primitive alternate phase. The flanking bundles are the intermediate and superposed phases. The development of the two earlier phases is suppressed only in the cotyledon lamina.

When the plumular vascular elements develop slightly later they are collateral and endarch, and are directly continuous with secondary elements formed by the cambium in the region of the hypocotyl. The inception of the plumular elements is thus marked by the emergence of only the more advanced type of vascular tissue, the more primitive phases being apparently arrested. As development continues, secondary vascular tissues rapidly assume prominence and a direct connection is established by them from stem, leaves, and branches, downward into hypocotyl and root. A continuous ectophloic siphonostele becomes the dominant conducting system through root and shoot. Even at the growing tip of the root where the primitive radial arrangement continues to form, secondary vascular elements laterally supplement the primary elements.

Thus the problem of continuity presented by the "transition" region is solved without great difficulty, for the primary vascular system of the root does not communicate directly with the more advanced vascular arrangement of the shoot. The seedling, with its temporary, leaflike cotyledons may be a recapitulation of the primitive ancestor of *M. sativa*. Certainly the superposed secondary system belongs to a later evolutionary period.

Summary

1. The primary vascular root system of *Medicago sativa* L. is triarch or, more rarely, tetrarch. Lateral roots arise directly opposite the xylem strands.

2. Triarchy in the root, when present in the lower portion, changes to tetrarchy in the hypocotyl region, although the fourth ray may be an abortive one.

3. Each cotyledonary trace is made up of a polar xylem strand and a part of each lateral xylem strand. Cotyledonary traces, as they pass into the cotyledons, are composed of triad bundles, the median portion being a polar protoxylem strand.

4. The median protoxylem strand of the cotyledonary trace disappears as the triad bundle approaches the cotyledon lamina. The two remaining flanking bundles fuse to form the collateral midrib bundle of the cotyledon blade.

5. The primary system of the root, hypocotyl, and cotyledons

forms a complete circulatory system of its own, independent of subsequent plumular development. It recapitulates the phases of evolutionary development as described by CHAUVEAUD in his phanerogamic cycle.

6. When the plumular elements develop, slightly later, they are collateral and endarch and are directly continuous with secondary elements formed by a cambium in the region of the hypocotyl.

7. The first plumular vessels to form are those which become traces to the first and second leaves. These leaves arise at alternate nodes above the cotyledonary node. Later, the formation of a complete cylinder of bundles in the stem obscures the priority of the emergence of these first trace bundles.

8. Secondary tissues early form a continuous conducting system through root and shoot. The primary tissues become imbedded in secondary tissues except at the tip of the root where they are laterally supplemented by secondary tissue.

9. The pattern of the vascular system in the stem is comparatively simple, being the result of three foliar traces alternating from node to node and of two branch traces supplying the branch which forms in the axil of each leaf.

10. Six stem bundles are principally involved in bud and leaf trace formation. These bundles partially lose their identity at the nodes because of anastomoses of all the bundles on the side of the stem where the leaf is forming.

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DEPARTMENT OF BOTANY
UNIVERSITY OF NEBRASKA
LINCOLN, NEB.

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MULTIPLE MALE CELLS IN CUPRESSUS ARIZONICA

CLIFTON C. DOAK

(WITH SEVENTEEN FIGURES)

Introduction

In spite of extensive work on the morphology of members of the Cupressineae, many uncertainties still remain. The following work on *Cupressus arizonica* was therefore begun in 1929. The present paper deals with a description of the male gametophyte.

MATERIAL AND METHODS.—The material was gathered in the spring of 1929 from a single tree, which is one of a small group growing in the grounds of Texas Agricultural and Mechanical College at College Station, Texas. The tree was approximately 25 years old and seemed to be in perfectly normal condition.

The nucellus, together with the inclosed female gametophyte, was carefully dissected from the hard seed coat while the material was submerged in 0.3 gm. molecular cane sugar solution, this having been successfully used by BUCHHOLZ (1) for the dissection of embryos of coniferous plants. Formalin acetic alcohol was used as a killing solution. The material was imbedded in paraffin, cut at 10μ, and stained in safranin and fast green, which gave good detail of nuclear and protoplasmic structures. Attempts made to section entire ovules were unsuccessful, due to the hard seed coats.

Investigation

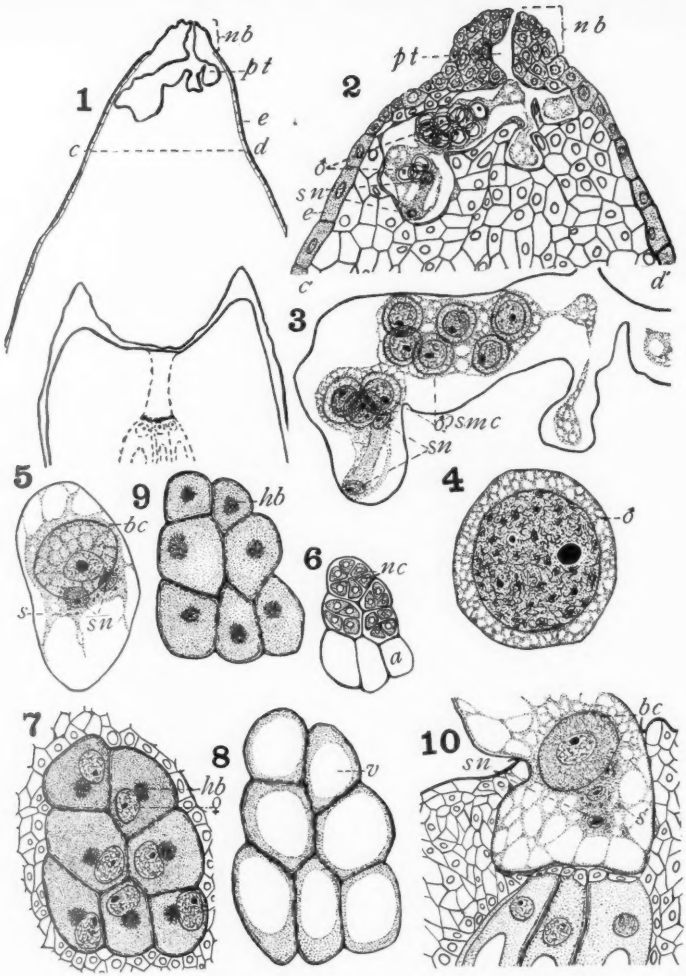
The nucellus is a conical structure, and is free from the integument to a point far below the top of the female gametophyte. The cells in this "nucellar beak" are smaller and have a greater protoplasmic content than have the cells in other regions (figs. 1, 2). The epidermal cover is discontinuous over the tip of the nucellus, and here the surface is somewhat irregular. The lower portion of the nucellus is covered with an epidermal layer which stains deeply with safranin (fig. 2 *e*). This layer is resistant to the action of the pollen tubes, and restricts their growth beyond the limits of the nucellus.

Pollen grains and pollen tube

The wingless pollen grains of members of the Cupressineae are shed in the microspore stage. There are never any prothallial cells. According to Coulter and Chamberlain (5), this condition is known in at least seven of the ten genera in this group, including *Cupressus*.

The first division of the microspore nucleus gives rise to the generative and tube nuclei. As shown by Coker (4), this takes place after the pollen is shed on the nucellus and before the tube is put out. Later a tube is formed and both nuclei enter it. At first the tube is small, but in the nucellus it usually widens rapidly and becomes evaginated and branched at various points, following a devious course (figs. 1, 2 *pt*). In a few cases the tube was found to be rather smooth and to follow an almost direct course; but even in such cases it widens greatly in the course of advancement, and upon entering the archegonial chamber soon broadens to fill this space completely (fig. 10). Within this tortuous and somewhat conical tube, the generative cell divides to form the stalk and body cells. The body cell increases enormously in size (figs. 5, 10, 16), and finally gives rise to the male cells. In most members of this genus these are limited to two which are equal in size and both functional. In the present material no very early stages were found. The youngest (June 13) shows the stalk and body cells already formed.

Many different developmental stages may be found at any given time, indicating considerable variation in the rate of growth and in the behavior of the different pollen tubes. Variation is also found in the time at which the division of the body cell takes place, as well as in the number of divisions which finally ensue. In some cases the undivided body cell was seen lying in the tip of a greatly swollen pollen tube which was already in contact with the necks of mature archegonia (fig. 10). In other instances, tubes in a similar position contained two equal, large, hemispherical cells which in every way resembled the two male cells so often described and figured for members of this genus. Another tube which had reached the archegonial chamber contained twelve curiously shaped "sperm cells" (figs. 14, 15 *b*). Often the division to form many male cells takes place long before the archegonial chamber has been reached. In one



FIGS. 1–10.—Fig. 1, longitudinal section of nucellus with ramified pollen tube; position of archegonia indicated in dotted lines below; ×162. Fig. 2, upper part of same, showing content of pollen tube built from serial sections; cytoplasmic content of male cells omitted; ×150. Fig. 3, more highly magnified view of best section from which fig. 2 was reconstructed; ×300. Fig. 4, more highly magnified view of one of male cells shown in figs. 2 and 3; ×1650. Fig. 5, cross-section of swollen end of pollen tube which filled archegonial chamber and was in contact with neck cells. Figs. 6–9, sections taken from different levels of same specimen; figs. 7 and 9 partly reconstructed from serial sections. Fig. 6, archegonial neck cells in face view. Fig. 7, cross-section of upper end of archegonial complex showing egg nuclei, each accompanied by a Hofmeister body or "asteroid." Fig. 8, median cross-section of same complex showing characteristic large vacuoles. Fig. 9, basal section of same; Hofmeister bodies or "asteroids" present in each archegonium. Fig. 10, longitudinal section of upper end of female gametophyte with pollen tube broadened to fill archegonial chamber; fig. 5–10, ×162.

case (figs. 1–3) a tube which had traversed less than one-third the distance to the archegonial chamber was seen to contain fourteen cells (possibly sperm mother cells).

Multiple fertilizations

Several types of evidence indicate that a great number of male cells from a single tube often participate in many separate fertilizations:

1. In spite of the fact that usually only one or two tubes enter an archegonial chamber, fertilization of the eggs in numerous archegonia of a single complex may take place almost simultaneously.

2. Often more than one male cell could be seen in a single archegonium (fig. 13), indicating that had the tube content been distributed differently more eggs could have been fertilized.

3. The contrast in size of the undivided body cell and its nucleus, in comparison with male cells participating in fertilization, would suggest that not half but a smaller fraction of this material goes into the construction of the functioning male cell. This may be seen by comparing the body cells shown (figs. 5, 10, 16) with the functional male cells (figs. 13, 15 *b*, 17).

4. There is some indication that the presence of one tube in position above the archegonial chamber tends to inhibit the entry of others, thus limiting the fertilizations in a single archegonial complex to the male cells from a single tube. A case was found in which one tube was in position above the archegonia, and another had traversed the tissues of the female gametophyte much as it had traversed the nucellus (fig. 15 *a*). A tube in this position would be at a great disadvantage, for there is evidence that the tube has not the enzymes necessary to penetrate the walls of the archegonia. For example, during normal entry, at which time the tube readily digests the neck cells, it leaves the upper walls of the archegonia intact. Even if one of these lateral tubes should effect an entry, it would gain access to only one or two of the archegonia. These lateral tubes were first seen by Hofmeister (**7**). He reported a case observed in *Taxus*, in which a number of such tubes twined about the archegonia in such a way as to shut off completely the source of nourishment to the archegonia.

5. Another indication of plurality of fertilizations by a single tube comes from the fact that, from data gathered for a forthcoming paper on the embryology of this species, I find that the number of zygotes per complex is in excess of what would be expected on a basis of two fertilizations per pollen tube.

6. As already pointed out, when there is more than one pollen tube growing within a single nucellus, their cellular contents are usually in widely different stages of development. Thus any eggs which remain unfertilized by the first tube would probably have to retain their ability to function for a considerable time while waiting for the maturity of the other tubes. There is abundant evidence that this is not the case, for after proembryo development has started within a part of the archegonia of an archegonial complex, the adjacent archegonia which do not contain proembryos rapidly disintegrate.

7. If the separate archegonia of a complex are served by tubes which vary considerably in their time of maturity, one would expect the resulting zygotes and embryos to vary proportionately as much as do the pollen tubes which initiate them. On the contrary, these structures are always in approximately the same developmental stages.

It seems safe to conclude, therefore, that the many male cells of a single pollen tube of *Cupressus arizonica* fertilize a number of eggs in the archegonia within an archegonial complex, and that in most cases the work of fertilization within a single complex is limited to the male cells from a single pollen tube.

Male cells

It has been pointed out that two hemispherical cells similar to those commonly found in other Cupressineae were seen in tubes already in contact with the necks of the archegonia, suggesting that the two functional male cells so commonly described for other species are found here also. However, these may represent cases of delayed division of the body cell. That such delay does occur is attested by the presence of undivided body cells even in pollen tubes which have already reached the archegonial chamber (figs. 5, 10). It is therefore impossible to say with certainty whether the two

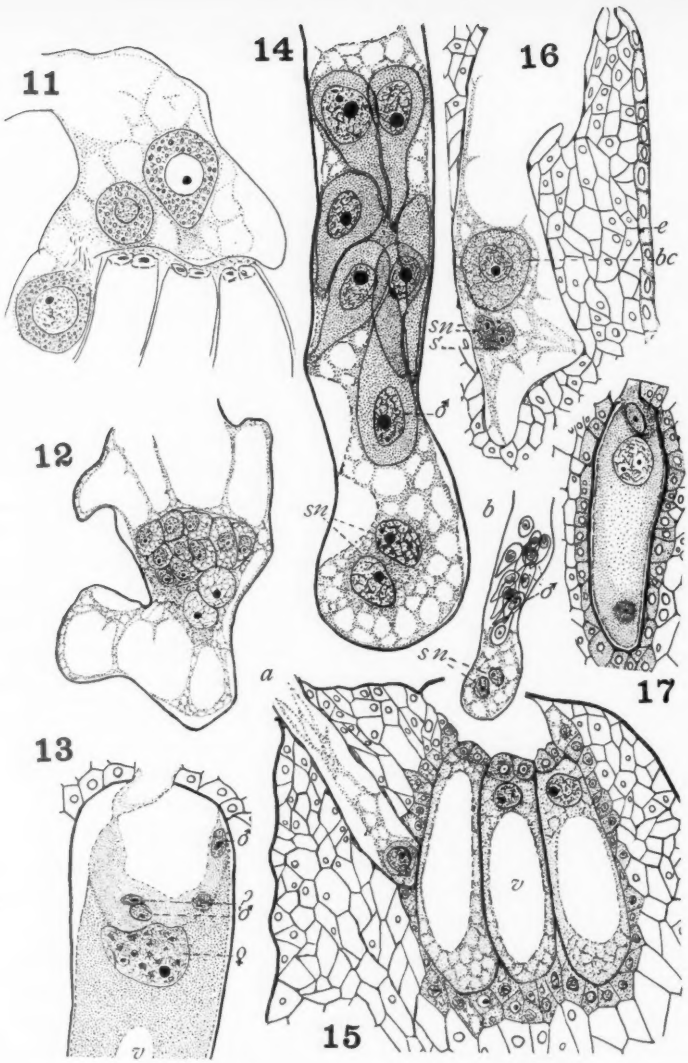
cells referred to participate in fertilization or first undergo further divisions.

In tubes which show the multicellular condition, the history of the male cells seems to indicate that the very large body cell (60–80 μ) divides rapidly and repeatedly to form a complex of from four to about fourteen male cells. At first the male cells are irregular in shape and lie together in a compact mass. It was in this condition that JUEL (**9**) delineated them in his figures, one of which is reproduced here as figure 12. JUEL shows this figure in series with a younger tube with undivided body cell and older tubes showing male cells which are slightly separated from each other and which have grown to approximately twice the size of the ones shown here. This increase in size makes the mature male cells as seen by JUEL in *Cupressus goveniana* approximately equal in size to those found in *C. arizonica*. Later the cells increase in size, round up, and separate from each other (figs. 2, 3). One tube was found in which there was a complex of male cells in which nearly all the cells were of a peculiar, elongated, pear-shaped form. Each had its own cytoplasm and was definitely bounded by a membrane (figs. 14, 15 *b*). This peculiar shape was not seen in earlier stages (figs. 2, 3) nor in male cells which had already entered archegonia (figs. 13, 17). Since this condition occurs here but once and not at all in the material of previous investigators, it is probable that this differentiation of the male cells into pear-shaped "sperm cells" is both evanescent and rare.

In addition to the tube and stalk nuclei and the male cells, the pollen tube contains a considerable amount of cytoplasm which is usually densest in the vicinity of the sterile nuclei (figs. 2, 3, 5, 10, 16). Frequently a few starch grains may be found in this cytoplasm (figs. 10, 16 s^1). When present, these grains are usually closely associated with the sterile nuclei. The starch is never abundant, as reported for many of the Cupressineae.

The number of archegonia in a complex ranges from three to ten, with six to eight occurring most frequently. A single pollen tube may come in contact with the necks of all the archegonia in a complex (fig. 10).

In entering the archegonia, the tube destroys or displaces the neck cells (figs. 13, 17). Just before entry, the mature egg nucleus



FIGS. 11–17.—Fig. 11 (from NICHOLS, fig. 94), "tip of a pollen tube which contained one small and two large male cells. One of the latter has already entered an archegonium." ×300. Fig. 12, from JUEL, fig. 2 (see text). Fig. 13, single archegonium in longitudinal section, showing presence of more than one nucleus from pollen tube; ×300. Fig. 14, best section from series from which fig. 15 *b* was reconstructed shown under higher magnification; seven of the twelve pear-shaped male cells ("sperm cells") shown; ×365. Fig. 15, longitudinal section of upper part of female gametophyte which has pollen tube (*a*) piercing the tissues; fig. 14 and the tube shown at *b* are from same specimen and represent a tube which had pierced the megaspore membrane as shown at *b;* ×127. Fig. 15 *a*, pollen tube which has pierced the tissues of the female gametophyte. Fig. 15 *b*, mature pollen tube with content of twelve male cells reconstructed from serial section; cytoplasmic content of male cells omitted; ×137. Fig. 16, longitudinal section of pollen tube with large body cell still undivided; ×325. Fig. 17, archegonium just prior to fertilization; only single male cell present; ×150.

is at the upper end of the archegonium and is surrounded by dense cytoplasm, which except for possible "HOFMEISTER bodies" is rather uniform in consistency. At this stage the large vacuole (fig. 15 *v*) is usually still present but is later filled by the addition of new cytoplasm to the archegonium. It has usually disappeared completely at the time of fertilization (fig. 17).

Discussion

HOFMEISTER, writing several years before the appearance of STRASBURGER'S work on fertilization in gymnosperms (**20**), did not clearly understand the rôle of the nucleus in fertilization. However, he evidently saw pollen tubes with both the usual two male cells and also others with the multicellular condition. He states, "In other cases the pollen tube contains four middle-sized or eight smaller, roundish cells without any firm membrane," and cites Pl. LXIV, fig. 3. This figure portrays a pollen tube in which seven nuclei are clearly shown. HOFMEISTER was near the truth as regards the fertilization of many eggs by the many male cells of a single pollen tube. He says, "In *Taxus* the impregnation of several corpuscula (archegonia) by the very widely expanded end of a single pollen tube is of very frequent occurrence and in *Juniperus* and *Thuja* it is the rule."

STRASBURGER speaks of the repeated division of one of the nuclei in the end of the pollen tubes of the Cupressineae and the distribution of the resulting cells over the necks of the archegonia. He states that in the Cupressineae a pollen tube fertilizes numerous eggs, and that a single nucleus is always seen above each archegonium before fertilization.

Preceded by these investigators, JUEL (**9**), working with *Cupressus goveniana*, was first to observe and completely interpret the plurality of male cells ("spermzellen") in the pollen tube of a conifer. In spite of the fact that other workers, notably NORÉN (**13**), NICHOLS (**12**), and SAXTON (**18**), have found evidences of irregularities in the number, manner, and time of male cell formation, it can be said that after nearly 30 years of more or less intensive work on the Cupressineae and closely related plants, JUEL'S work has stood, until now, unconfirmed. In speaking of the work of JUEL, COULTER and CHAM-

BERLAIN say that, "At present no conclusions are safe in regard to such rare occurrences."

Even as early as 1907 the view that JUEL'S findings represented interesting abnormalities had already begun to be current, and this view has frequently been expressed since that time. LAWSON (11), after having described the two male cells as he found them in *Libocedrus decurrens*, points out that almost exactly the same conditions obtain in regard to the two species of *Cupressus* examined by him. He then adds that, "From LAND'S (10) account of *Thuja*, COKER'S (3) account of *Taxodium*, and my own observations of *Cryptomeria*, *Libocedrus*, *Thuja*, *Cupressus*, and *Chamaecyparis* there seems to be a striking uniformity throughout the Cupressineae in regard to the history of the male gametophyte." If LAWSON were writing today, he could strengthen his argument in favor of the normality of two equal male cells by adding to the above list, OTTLEY'S (14) account of *Juniperus communis* and *J. virginiana*, NICHOL'S (12) account of *J. communis*, and possibly others. For the Cupressoideae and Callitroideae he could add SAXTON'S work with *Widdringtonia cupressoides* (16), *Callitris* (17), *Actinostrobus pyramidalis* (18), and *Tetriclinis articulata* (19). In this body of work, however, at least three writers, NORÉN, NICHOLS, and SAXTON, reported having seen more than two male cells in a single pollen tube. Although little was made of these observations, the evidence is sufficient to show that this condition did occasionally occur in their materials, and that the male cell number is not rigidly fixed at two in all members of the Cupressineae.

LAWSON seems either not to have been familiar with the works of HOFMEISTER (not cited) and STRASBURGER, or else unwilling to accept their obvious implications, for in defending the position that the organization of two male cells of equal size is a constant character of the Cupressineae, he says that, "The only exception which has so far been recorded is that of *Cupressus goveniana*, in which JUEL described the body cell as giving rise to a complex of cells."

The present study confirms in nearly every detail the work of JUEL on *Cupressus goveniana*, and at the same time adds some significant stages not seen by him. The most prominent of these new features is the partial differentiation of certain of the male cells into "sperm cells."

In seeking to account for the peculiar shapes assumed by these cells (fig. 14), their similarity in outline to certain zoospores or to the sperm cells of cycads and *Ginkgo* recalled HOFMEISTER'S reference to the possibility of sperm cells in the pollen tubes of the Coniferae.

A search of the literature was made to see whether male cells with similar shapes had been seen before. In a few cases there is a suggestion of such a thing; but in most of these the elongated shapes are such that they could be accounted for by the recent mutual pressure of the newly formed hemispherical male cells which had simply not yet fully rounded out. SAXTON (**19**) reports that the male cells of *Tetraclinis articulata* retain their hemispherical shapes even after long separation from each other. NICHOLS (**12,** Pl. XVI. fig. 94, reproduced here as fig. 11) shows a pollen tube of *Juniperus communis* with three male cells, which according to him represents an abnormal condition for this species. Two of these cells are slightly elongated. It would appear that NICHOLS saw a suggestive shape in the male cells of a pollen tube, which also had supernumerary male cells. Perhaps it is a mere coincidence that these were in a tube which had more than the usual number of male cells, and which had reached the same stage in its ontogeny as the tube which contained the pear-shaped cells in my material. In speaking of the matter of cilia, NICHOLS states:

> The demonstration of blepharoplasts in the body cells of the Ginkgoales and Cycadales suggests the possibility that some traces of cilia-forming organs might exist among the Coniferales and Gnetales. A careful study, however, of a large number of body cells in *J. communis depressa*, in all stages of development, has failed to reveal any structures which appear definitely homologous with blepharoplasts.

JUEL'S figures were all taken from material which was either too young or too closely compacted to show these elongated shapes.

HOFMEISTER predicted the finding of swimming sperms in the pollen tubes of the gymnosperms. HIRASÉ (**6**) partly fulfilled the prophecy by announcing their discovery in *Ginkgo*. IKENO (**8**) added *Cycas revoluta*, and WEBBER (**21**) added *Zamia integrifolia*. HOFMEISTER at the time of his prediction, however, was concerned neither with *Ginkgo* nor with a cycad, but with various higher conifers, especially the members of the Cupressineae. Although no claim is made that cilia have been found, the evidence is such that new

hope is raised for the ultimate fulfillment of HOFMEISTER'S prediction.

The pollen tube of the angiosperms, because of the phenomenon of double fertilization, is not exactly comparable with that of the gymnosperms. The fact remains, however, that the former contains two functional male nuclei while in many gymnosperms the number is reduced to one. A second nucleus is present in various stages of development but is non-functional. Among many of the Cupressineae, the functional number seems to be more or less fixed at two. In many cases the stabilization is not complete and members of the Cupressineae develop tubes with more than two male cells. In certain still less stable forms, notably *C. goveniana* and *C. arizonica*, two male cells may be produced occasionally, but more regularly they produce a complex of several which in some cases become differentiated into "sperm cells."

The question arises as to whether these characters in *Cupressus* are palingenetic or coenogenetic. JUEL favors the palingenetic view. He holds that, since the gymnosperms in general can accomplish multiple fertilizations simply by growing a plurality of pollen tubes, and since only one embryo can come to maturity, multiple fertilizations through plurality of male cells would therefore have no survival value, and could not have arisen out of a form which produced but two male cells. JUEL overlooked the possible advantages of internal embryonic selection, or of secretory effects of multiple suspensors which BUCHHOLZ (1) later pointed out might account for the prevalence of polyembryony among conifers. JUEL concludes that we must assume for the Cupressineae a phylogenetic line which has preserved the multicellular condition. He holds that the Cupressineae and the other conifers split off very early from a common ancestor and have developed in parallel lines.

COULTER and CHAMBERLAIN take the opposite view. They state:

> While phylogenetic continuity of multiple sperms is safely claimed for *Microcycas* (CALDWELL 2), no such claim could be maintained for Cupressineae.

In speaking of a single versus a plurality of sperm mother cells they say,

> It is interesting to note that the very few instances of a greater number of sperm mother cells occur in a group characterized by its retention of ancient features (Cycadales), and in another group characterized by its very modern features (Cupressineae).

Phylogenetically an organism, or group of related organisms, is made up of an assemblage of many evolving structures which, despite the fact that the parts may not evolve at equal rates, for questions of phylogenetic position must be considered in their entirety. When it is so considered, the group making up the Cupressineae is unquestionably advanced, not only in regard to the sporophyte and female gametophyte, but in regard to the vegetative tissue of the male gametophyte itself. The writer is therefore inclined to the position that the cupressinean assemblage is advanced, and that the male cell complex found in certain members of the Cupressineae is a reversion which was made possible in a limited number of forms by the accidental sequence of evolutionary development between two structures which were somewhat interdependent, that is, the archegonial complex and the male cell complex. It would seem that in the female gametophyte the condition of scattered archegonia is primitive and the archegonial complex advanced, and that for the male gametophyte a plurality of sperm cells is primitive and the reduced number advanced. In ancient forms which existed before the advent of the pollen tube as a sperm-conveying mechanism the sperms moved from the spermagonium to the archegonium through their own motility. Under such conditions, in spite of the scattered archegonia, the sperms from a single spermagonium could readily fertilize the eggs in widely separated archegonia. Without claiming the Cordaitales as a direct ancestor for the Cupressineae, attention is called to the pollen grain of *Cordaianthus grand'euryi* with its many hundred cells (as figured by RENAULT **15**) as probably representing this level in evolutionary development. The establishment of the pollen tube as an organ of transfer of male cells to the archegonia rendered obsolete all male cells in excess of one, and eliminated the necessity for cilia. Evolutionary reduction set in, and certain forms, such as *Taxus*, *Pinus*, *Abies*, etc., in which the archegonia have remained permanently separated, show the evolutionary reduction in the male gametes as it approaches its final culmination in a single functional male nucleus. In certain forms, however, represented by *Juniperus*, *Biota*, and *Thuja*, in which the female gametophyte produces an archegonial complex, the male nuclei of the pollen tube could fertilize a plurality of eggs. This condition favored those pollen tubes which contained the largest number of male cells, and brought about a

return to, and a re-establishment of, the primitive condition of multiple male cells. In most Cupressineae the pollen tube seems to have been stabilized at two male cells before the formation of an archegonial complex. In *C. goveniana* and *C. arizonica* the pollen tube, by virtue of its greater variability, reverted to the habit of forming a male cell complex. Since the pollen tube is retained as a gamete-conveying organ, it is not to be expected that the reversion will extend to the fixation of ciliated sperms as a regular habit; but that these, if seen at all, will represent the extreme of the atavistic tendency which for lack of survival value has not, and possibly will not, become permanently re-established.

Summary

1. The ontogeny of the male gametophyte of *Cupressus arizonica*, with the exception of the early stages, is reported.

2. There is great irregularity in the behavior of the pollen tube and in the time of its development.

3. Some evidence is cited to support the view that two equal, functional male cells may occasionally occur.

4. In most cases a pollen tube develops a complex of several male cells, and occasionally these are differentiated into "sperm cells" with distinctive shapes, suggesting possible motility.

5. Although blepharoplasts were not seen and the evidences of cilia were not conclusive, the possibility is suggested that ciliated sperms may be found among the higher conifers. The complete return to ciliated sperms as a regular habit is not regarded as likely, but may occur as representing the extreme of an atavistic tendency.

6. Evidence is given to support the view that the many male cells of a single pollen tube serve to fertilize a number of different eggs in a number of separate archegonia of the complex.

7. The male-cell complex is considered to be a reversion. The trend toward reduction was reversed when the development of an archegonial complex made possible multiple fertilizations from a single pollen tube.

I am indebted to Dr. J. T. BUCHHOLZ, of the University of Illinois (formerly of the University of Texas), for having pointed out

the desirability of this study and for many valuable suggestions during its progress. I am also indebted to Mr. U. R. GORE, of Texas A. and M. College, for having gathered the material.

AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS
COLLEGE STATION, TEXAS
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ECOLOGICAL ASPECTS OF TRANSPIRATION

II. PIKE'S PEAK AND SANTA BARBARA REGIONS: EDAPHIC AND CLIMATIC ASPECTS

CHARLES J. WHITFIELD

(WITH SIX FIGURES)

Introduction

The first part of this research (**5**) dealt with the functional responses of plants and the measurement of physical factors in the main climatic associations of the Pike's Peak region, Colorado (**6**). In addition the vegetation was briefly described, and details of the phytometer and instrumental methods employed in these investigations discussed. The present paper deals with the edaphic aspects of the Pike's Peak region, and the edaphic and climatic relations of Santa Barbara County, California.

In Santa Barbara County, investigations were made in the coastal sagebrush and the coastal chaparral associations, and in the sand dunes along the coast (**1**, **7**). The conspicuous dominants of the coastal sagebrush are *Artemisia californica*, *Salvia mellifera*, *S. leucophylla*, *S. apiana*, and *Eriogonum fasciculatum*. The principal chaparral dominants are *Adenostoma fasciculatum*, *Quercus dumosa*, *Cercocarpus parvifolius*, and species of *Arctostaphylos* and *Ceanothus*. The most abundant dune species are *Lupinus chamissonis*, *Abronia maritima*, *Franseria bipinnatifida*, *Solanum douglasii*, *Heterotheca grandiflora*, and *Convolvulus soldanella*.

The climate of Santa Barbara County is equable throughout the year, excessive heat and cold being very rare. The average seasonal rainfall from 1925 to 1930 was 15.68 inches, and was confined to the winter and spring months.

Results

PIKE'S PEAK REGION

FIRST SERIES.—Plants of *Helianthus annuus* were used as phytometers for this series, which was conducted at the Alpine Laboratory. On the basis of sunlight representing 100 per cent, light intensities

on a clear day in the shade habitat were as follows: 9 A.M. 0.992; 12 M. 1.125; and 3 P.M. 0.694 per cent. Air temperatures were approximately 6° higher in the sun habitat, the average day and night temperatures being 73° and 56° F. in the sun garden and 65° and 53° F. in the shade habitat. Soil temperatures taken from thermometers inclosed in the phytometers averaged 53° in the sun garden and 52° F. in the shade. The temperature of the garden soils was approximately 57° in the sun garden and 55° in the shade (table I).

TABLE I

FIRST EDAPHIC SERIES, JULY 10–AUGUST 3, 1929 (CF. FIG. 1)

	TRANSPIRATION PER 100 SQ. CM.																	
	7/11	7/12	7/13	7/14	7/15	7/16	7/17	7/18	7/19	7/20	7/25	7/29	7/30	7/31	8/1	8/2	8/5	
Sun	19.0	19.0	23.0	14.0	10.0	7.4	9.0	14.0	5.2	12.0	51.0	54.0	9.6	5.1	3.7	11.0	32.0	
Shade	6.9	7.0	7.0	4.2	3.6	4.2	4.4	3.9	1.3	1.7	19.0	13.0	2.6	3.5	.9	2.8	4.5	
	AVERAGE STEM DIAMETER (MM.)																	
Sun	4.4	4.5	4.6	4.6	4.9	4.9	....	4.8	.....	.....	5.9	6.6	.....	.....	.....	.....	.....	7.1
Shade	4.3	4.5	4.6	4.7	4.7	4.7	....	4.7	.....	.....	5.4	5.8	.....	.....	.....	.....	.....	5.7
	LEAF AREA (SQ. CM.)																	
Sun	142	161	174	187	201	215	216	.....	.....	244	334	380	.....	.....	.....	.....	.....	493
Shade	119	140	161	182	195	189	190	.....	.....	215	270	301	.....	.....	.....	.....	.....	339

The experiment was started July 10 and ended August 3, 1929. Weighings were made at 5 P.M. daily from July 10 to 21, on July 26, and daily from July 30 to August 3. Leaf areas were taken at regular intervals. On August 3 the dry weight of the plants was determined.

Transpiration determined in three sun and shade habitats gave similar results (fig. 1).

SECOND SERIES.—Plants of two species, *Mertensia sibirica* and *Helianthus annuus*, were employed as phytometers for the final edaphic series. A slight rain of 0.02 inches was recorded during the experiment. Cloudy conditions prevailed during the afternoons of the nineteenth and twentieth, the mornings of these two days being clear or with intermittent clouds. The day of the twenty-first

was clear, while the morning of the twenty-second was intermittently cloudy. No leaf measurements were made, the water losses being determined on the bases of standardizations made before and after the series. Light readings were made in the shade area with a hand-photometer (table II).

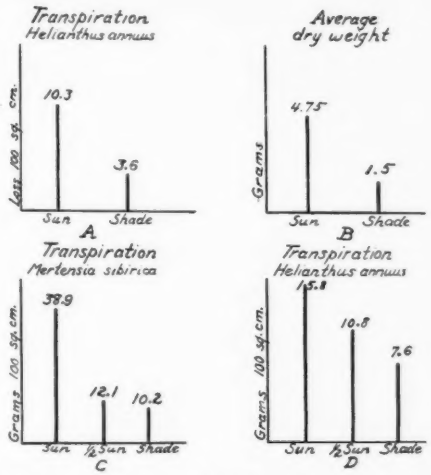

FIG. 1.—First series: *A* and *B*, July 10 to August 3, 1929 (cf. table I); *C*, July 5–7 1930; *D*, July 23–28, 1930.

TABLE II

LIGHT PERCENTAGES OBTAINED WITH HAND-PHOTOMETER

	8/19/31			8/20				8/21							
	9 A.M.	12 M.	3 P.M.	6 A.M.	8	10	12 M.	6 A.M.	8	9	10	12 M.	2 P.M.	4	6
Percentage	2.2	17.5	2.7	0.3	1.3	3.3	20.0	1.1	1.1	1.6	1.8	20.0	8.0	4.1	0.6

Average light intensities, determined over a period of four hours, were approximately 0.91 cal./cm.²/min. in the sun and 0.025 cal./cm.²/min. in the shade. The maximum intensity was 1.40 in the sun and 0.084 cal./cm²/min. in the shade, the minima being 0.382 and 0.012. Light intensities were measured by means of a pyranometer, which measures intensity of the total radiation falling

TABLE III

SECOND SERIES; TRANSPIRATIONAL AND INSTRUMENTAL DATA

		8/19					8/20							8/21							8/22		
		10 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	6 A.M.	8 A.M.	10 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	6 A.M.	8 A.M.	10 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	6A.M.	8 A.M.	10 A.M.
Transpiration, H. annuus	Sun	.....	14.3	10.0	7.0	2.3	1.7	6.7	11.0	13.3	7.0	5.3	4.0	4.3	4.3	14.0	18.3	14.0	14.0	8.5	4.8	9.1	10.0
	Shade	.....	4.8	4.0	1.7	1.0	1.7	1.0	2.0	4.0	4.3	2.7	0.7	2.0	1.3	3.3	6.0	5.5	2.5	2.0	1.0	2.0	2.0
Transpiration, M. sibirica	Sun	.....	13.3	10.2	7.1	1.8	4.9	5.8	11.5	13.8	11.5	5.3	4.4	5.3	3.8	13.3	16.9	16.0	12.4	6.21	5.8	7.5	9.8
	Shade	.....	6.0	4.5	2.0	1.0	1.0	2.5	3.0	4.0	4.0	2.0	0.5	3.5	1.0	5.0	6.0	8.0	3.0	2.5	2.5	1.5	3.5
Outside temperature, 6 in. high	Sun	76	74	65	71	63	52	73	72	76	69	70	64	53	72	77	83	80	76	70	55	75	77
	Shade	63	66	60	64	58	49	59	66	70	66	63	59	51	62	69	72	72	70	64	54	64	69
Soil temperature at 6 in.	Sun	66	72	74	72	70	59	61	68	70	72	70	69	59	61	67	75	79	77	72	61	63	69
	Shade	56	52	53	54	54	51	51	52	53	54	54	56	50	50	52	55	59	59	59	52	53	54
Soil temperature in cans	Sun	.....	84	84	78	73	59	59	65	72	75	73	73	59	58	62	72	80	81	81	61	61	67
	Shade	.....	74	71	65	62	52	52	54	56	57	58	58	52	52	54	57	61	62	63	54	54	56
Humidity, psychrometer	Sun	50	45	59	41	59	49	37	41	29	31	37	45	49	33	29	23	44	17	46	47	32	29
	Shade	52	66	80	67	75	88	56	54	46	54	69	80	88	57	55	43	31	31	67	78	68	41
Saturation deficit	Sun	3.74	4.30	2.52	4.56	2.52	2.80	4.87	4.56	6.44	5.87	4.87	3.73	2.47	5.52	6.44	8.16	5.93	7.76	3.78	2.75	6.16	6.23
	Shade	3.25	2.54	2.28	1.96	1.39	0.047	2.98	3.33	4.45	3.33	2.10	1.11	0.49	3.01	3.59	4.85	6.06	5.87	2.09	0.995	2.10	4.56
Piché evaporimeter, cc. lost	Sun	.....	0.9	1.8	0.9	0.7	1.5	0.9	1.7	2.3	1.8	0.9	.....	.....	0.9	1.9	2.8	2.7	2.6	1.4	3.9	1.1	1.6
	Shade	.....	0.6	0.8	0.4	0.4	0.4	0.5	0.7	1.0	0.9	0.5	0.3	1.6	0.4	0.8	1.1	1.2	1.2	0.9	1.9	0.4	0.6
Evaporimeter, inches lost	Sun	.....	0.08	0.07	0.08	.....	.....	.....	0.01	0.09	0.06	0.05	.....	.....	0.18	0.03	0.10	0.15	0.07	0.02	0.20	0.09	0.04
	Shade	.....	0.02	0.02	0.01	0.0	0.03	0.01	0.01	0.01	0.03	0.0	0.01	0.03	0.03	0.01	0.01	0.04	0.03	0.02	0.04	0.03	0.01
Anemometer, gain in miles	Sun	.....	5.6	6.0	3.3	1.2	24.0	4.4	3.4	5.7	4.2	3.8	0.5	23.4	4.6	3.4	4.0	4.1	5.8	2.9	24.8	4.7	2.9
	Shade	.....	.....	0.6	0.4	0.1	0.1	0.8	0.8	1.5	0.6	0.6	0.0	2.5	0.3	0.2	0.4	0.5	1.1	0.3	1.1	0.2	0.2

on a horizontal surface. Total water losses with plants of *Mertensia sibirica* as phytometers for the 4-hour period were 8.2 gm. in the sun and 1.6 gm. in the shade. Air temperatures varied from 62° to 76° in the sun and from 59° to 71° in the shade; while relative humidities averaged 49 and 52 per cent in the sun and shade respectively. These data indicate that radiant energy is an important factor in transpiration.

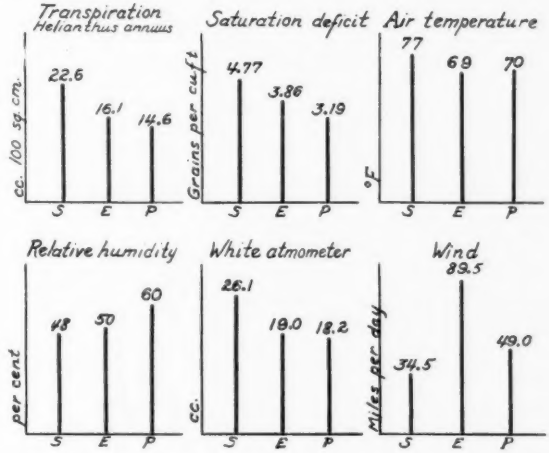

FIG. 2.—Third series, April 16–17, 1931: *S*, Santa Barbara; *E*, dunes exposed; *P*, dunes protected.

The principal experiment was started at 10 A.M., August 19 and closed at 10 A.M., August 22, 1931. Weighings and measurements were made every 2 hours from 6 A.M. to 6 P.M. (table III).

The stomatal conditions of the plants in the two habitats were practically similar.

SANTA BARBARA REGION

THIRD SERIES.—This experiment was conducted at three stations, one located in the coastal sagebrush association, and two in the sand dunes. One of the dunes stations was on an exposed knoll with a high wind velocity, while the second was in a protected area which was surrounded by *Lupinus chamissonis* and *Solanum douglasii*. The temperature, humidity, and saturation deficit data are based on

noon readings. The experiment was started at 6 A.M., April 16 and closed at 6 P.M., April 17, 1930 (fig. 2).

FOURTH SERIES.—This series was conducted during the week of March 2–9 at the Santa Barbara and the protected dunes stations. Plants of *Helianthus annuus* were employed as phytometers. Read-

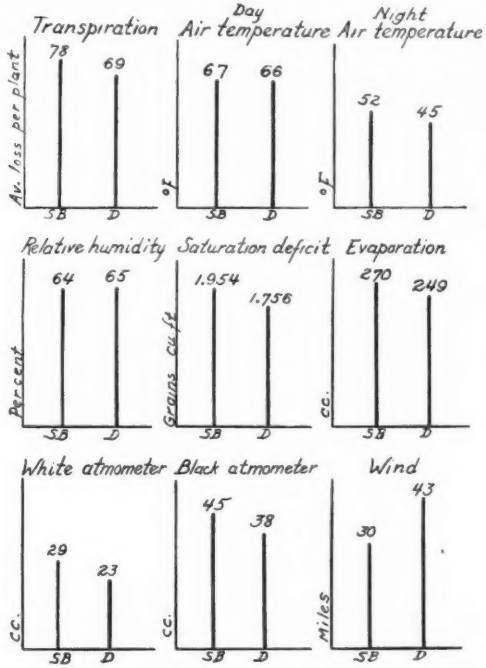

FIG. 3.—Fourth series, March 2–9, 1931

ings were made daily. During this period, water loss at the mountain station was approximately the same as at the Santa Barbara station (fig. 3).

FIFTH SERIES.—The third experiment at Santa Barbara was conducted April 7–10 at the mountain and the Santa Barbara stations. The mountain station was located at an elevation of 3000 feet. Plants of *Helianthus annuus* were used as phytometers. Weighings were made daily (fig. 4).

Discussion

Pike's Peak region

Radiant energy seems to be the most important factor between sun and shade habitats. Other factors, such as air and soil tempera-

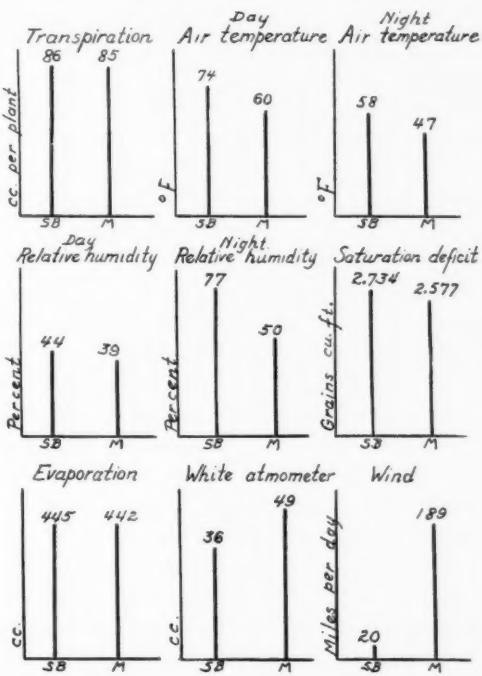

FIG. 4.—Fifth series, April 7–10, 1931: *SB*, Santa Barbara; *M*, mountain

tures, relative humidities, and saturation deficits, do not show the great differences that radiant energy does, and are probably therefore of less importance.

There were greater transpirational losses, growth, and dry weights in the sun than in the shade habitat. These edaphic studies also indicate a relationship between growth and transpiration.

There was a gradual decrease in transpiration from the beginning to the end of the experiment (table I). This is in agreement with

MAXIMOV's results (4). The results secured here with *Helianthus annuus* and *Mertensia sibirica* in edaphic conditions are in general accord with those of CLEMENTS and GOLDSMITH (2, 3).

The vegetation of sun and shade habitats varies according to the density of the shade. With lower light intensities, only tolerant species are able to grow. In the deeper shade of the montane zone most of the species are reduced in size, many not flowering; while in more moderate shade conditions the species are larger. The tolerant climatic species, for instance *Pseudotsuga mucronata*, are able to grow and reproduce in shade conditions, and consequently are to be found dominating the shady north slopes and ravines; while the dry south slopes are occupied by more intolerant species, for instance, *Pinus ponderosa*.

SANTA BARBARA REGION

Transpirational studies were conducted in the Santa Barbara region primarily to determine the relationships between the dune vegetation and that of the coastal chaparral.

Water losses were found to be lower in the dunes than in the coastal chaparral. Saturation deficits, air temperature, and evaporation were also greater in the coastal chaparral. Relative humidities and wind were higher in the dunes. These facts seem to indicate that the coastal sand dunes are not so xeric as is supposed. The probable explanation of the dune vegetation lies in the edaphic conditions rather than in the climatic, the presence of the sand being the important factor.

Transpiration, saturation deficit, evaporation, and wind were greater in the exposed than in the protected dune area. Relative humidity was higher in the latter.

In these experiments, as in the preceding ones, the ratios between transpiration and any one factor showed closer relationships with air temperature and relative humidity.

Experiments conducted in the coastal chaparral at elevations of 200 and 3000 feet showed transpiration at the two stations to be practically similar. Environmental factors at the two stations were variable. The losses from the white atmometer cup plainly show the effect of wind.

These results seem to indicate a definite correlation between factor, function, and vegetation. The sand dunes, although really more

mesic than the chaparral climatically, appear xeric, owing to the growth of the vegetation in a sandy soil. Throughout the chaparral association the total effect of all climatic and edaphic factors produces similar results in functional and vegetational responses, even though the individual factors vary.

Evaluation of the various factors

A second series of experiments was conducted to determine the chief external factor or factors influencing transpiration and the effect of each factor.

GENERAL.—Initial experiments were conducted in the alpine tundra at an elevation of 12,100 feet. Thirty sunflower plants were used in each of these experiments. The plants were potted in glasses, sealed, and placed in openings cut in the Celotex lids of boxes. The sunflowers were weighed hourly, and soil and air temperatures were recorded at each weighing.

The results indicated that a low soil temperature is an important factor in limiting transpiration, since a soil temperature of approximately 37° F., even with a rising air temperature, caused the plants to wilt. As the soil temperature rose, the transpirational curve seemed to follow more closely the air temperature curve. Later, however, when a cloud obscured the sun, the transpirational curve dropped more rapidly than did the air temperature, indicating that radiant energy is also an important factor in transpiration.

SOIL TEMPERATURES.—Experiments to determine the effect of various soil temperatures on transpiration were conducted at the Alpine Laboratory during the summers of 1930 and 1931.

Three conditions, cold, intermediate, and warm, with respective temperatures of 36°, 51°, and 113° F., were used for the initial experiment. Thirty standardized sunflower plants were divided into three groups of ten each, weighed and placed in the conditions already described. When reweighed, the batteries showed the following losses: cold, 19.0; intermediate, 46.2; and warm, 53.3 gm. The results show a rapid rise of the transpirational curve with a comparatively low increase of soil temperature, and a flattening off of the transpirational curve with high soil temperatures. These results indicate, as does the initial experiment conducted in the alpine tundra, that when soil temperatures get above 40° F., they are not as im-

portant in influencing transpiration as they are below this temperature.

This experiment was repeated and others conducted to check further the results obtained. The data for a typical experiment are shown in figure 5.

Four different soil temperature conditions: cold, two intermediate, and warm, were used. The cold temperatures were secured by packing the phytometers in a Celotex-lined box with ice, water, and

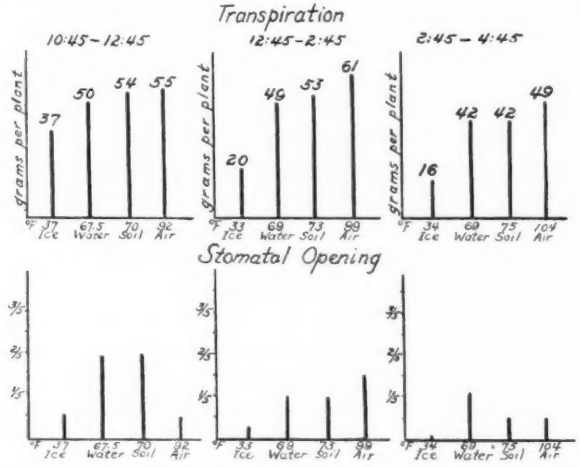

FIG. 5.—Graph of transpiration, soil temperature, and stomatal opening

salt. For the next higher soil temperature, the plants were placed in a box with water from a mountain stream running through it. The third condition was secured by placing the containers in a large box filled with soil. The plants were thus on a level with those in the other conditions. The highest soil temperature was secured by placing the phytometers on the surface of the soil, exposing them to the air temperature. Lids were constructed for each box in order to prevent a direct effect of the ice, water, and soil on the immediate environment of the various batteries. The sky conditions for this experiment were ideal, being practically cloudless.

The stomata of the plants of the various batteries were examined. Standardized plants of *Mertensia sibirica* were used as phytometers

in this experiment. The data shown on the graph are averages for the period.

These results further strengthen the previous conclusion as to the effect of soil temperature on transpiration and the flattening of the latter curve with higher soil temperatures. In addition there is seen to be a close correlation between stomatal opening and water loss. The plants in the ice-box began to wilt toward the end of the first period, and were all wilted by the middle of the second period. In the beginning the stomata of these plants were one-fifth open to closed. With the plants remaining in the wilted condition, the stomata became more tightly closed, and the losses consequently diminished. Further proof that low soil temperature is the predominant factor is derived from the fact that with the same stomatal opening, plants having soil temperatures of 37° and 92° F. gave losses of 37 and 55 gm. respectively (fig. 5). That a reduced stomatal opening limits transpiration is also shown by a comparison of the losses of the plants in the warm and intermediate conditions of the three periods. The relationship of soil temperature, stomatal opening, and transpiration is still further shown in the third period. In the first two periods the plants in the soil had been losing more than those in the water-box with similar stomatal openings. In the last period the stomata of the plants in the soil started to close before those of the plants in the water-box, consequently limiting the water losses, which were equal for the soil and water conditions for this period.

The plants were restandardized at the end of the experiment. The results indicate that the plants were not physiologically affected by the different conditions of the experiment.

RADIANT ENERGY.—Other experiments were conducted to determine the effect of radiant energy on transpiration. An equatorial mounting 5 feet tall with a cardboard top 3 feet square was constructed. The mounting was tall enough to permit free air movement, and could be adjusted to keep the plants always protected from the sun.

Standardized *Mertensia sibirica* plants were used for the experiment. Light readings were made with the pyranometer in the shadow cast by the mounting and also in the open. Air tempera-

tures, leaf temperatures, and relative humidity readings were also taken.

Leaf temperatures were approximately 3.5° F. higher in the open than under the mounting.

TABLE IV

RELATIONSHIP BETWEEN RADIANT ENERGY AND TRANSPIRATION

	AVERAGE H_2O LOSS PER PLANT (GM.)	AVERAGE LIGHT INTENSITY (CAL.)	AVERAGE AIR TEMPERATURE (° F.)	AVERAGE RELATIVE HUMIDITY (PER CENT)
Sun	24.7	0.911	70.4	49.6
Shade	9.7	0.096	70.2	49.0

TABLE V

EFFECT OF RADIANT ENERGY ON TRANSPIRATION

DATE (1931)	SPECIES	CONDITION	TIME	LOSS (GM.)	RADIANT ENERGY	TIME	LOSS (GM.)	RADIANT ENERGY CAL./CM².MIN.
8/13	Mertensia sibirica	Open	6–9	24.0	0.720	9–12	37.0	1.370
		Cheesecloth	"	21.0	0.280	"	31.0	0.610
		Muslin	"	17.0	0.150	"	27.0	0.340
8/14	Mertensia sibirica	Open	3–5	30.0	0.720	.....	.....	.....
		Cheesecloth	"	21.0	0.250	.....	.....	.....
		Muslin	"	19.0	0.180	.....	.....	.....
8/17	Mertensia sibirica	Open	10–12	24.0	1.270	12–2	24.0	1.150
		Cheesecloth	"	20.4	0.478	"	14.8	0.452
		Muslin	"	17.4	0.288	"	11.0	0.244
	Helianthus annuus	Open	"	27.0	1.270	"	30.0	1.150
		Cheesecloth	"	21.0	0.478	"	15.0	0.452
		Muslin	"	20.0	0.288	"	16.4	0.244
8/18	Helianthus annuus	Open	"	20.0	1.120	"	20.0	1.420
		Cheesecloth	"	17.0	0.428	"	17.0	0.453
		Muslin	"	18.0	0.274	"	16.0	0.354

The results show a higher light intensity and a higher water loss in the sun than under the mounting. The ratios for radiant energy and water loss for the two conditions were 9.5 and 2.35 gm. respectively. The fact that the ratio for radiant energy is much higher than that for transpiration indicates that above a certain point radiant energy ceases to have much effect on transpiration.

As table IV shows, air temperature and humidities were similar in the two conditions, but leaf temperatures were slightly higher in the open.

The results of later experiments conducted with different light intensities indicated that radiant energy is one of the principal factors affecting transpiration.

SOIL MOISTURE.—In order to check the relation of different soil moistures to transpiration, sunflower plants were grown in a uniform soil and standardized. The plants were then divided into three groups, one set allowed to approach the wilting coefficient (or a low

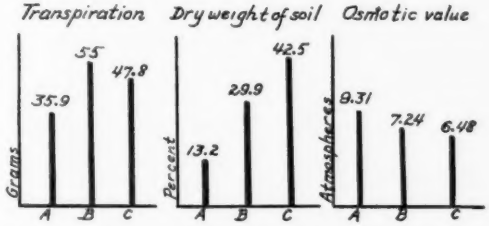

FIG. 6.—Graph of transpiration, percentage of dry weight of soil, and osmotic concentrations of the plant in atmospheres of pressure.

water content), another brought to a medium content, and the third given a high holard. The experiment was conducted for a 5-day period, at the end of which time water contents were figured on the basis of dry weight and the osmotic values were determined.

Water loss apparently increases with the water content until the optimum conditions are reached. In this experiment the highest water content gave a water loss below that of the medium, owing undoubtedly to poor aeration. Osmotic values showed a constant decrease with increased soil moisture (fig. 6).

Summary

1. Radiant energy seems to be the most important factor between sun and shade habitats. Shade vegetation does not respond to any one factor, however, but rather to all of the environmental conditions.

2. Although individual factors varied, the environmental complex

at two different stations produced similar functional responses in the coastal chaparral association.

3. The xeric character of the vegetation of the sand dunes is due to an edaphic rather than to a climatic condition.

4. Soil temperature, below approximately 37–39° F., is an important factor in limiting transpiration. Water loss seems to be increased by higher soil temperatures up to a certain point where the curve flattens off. The effect of different soil temperatures requires further study.

5. Transpiration was found to be highest in soils of medium water content, next in saturated soils, and lowest in soils with low water contents.

6. Osmotic values showed a constant decrease with increased soil moisture.

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AGRICULTURAL BUILDING
UNIVERSITY OF ARIZONA
TUCSON, ARIZ.

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CHEMICAL COMPOSITION OF ASPERGILLUS NIGER AS MODIFIED BY ZINC SULPHATE[1]

NANDOR PORGES

Introduction

The ubiquitous black spore-bearing fungus, *Aspergillus niger*, produces a number of organic acids (**1, 4, 9, 11**) which vary with the methods of cultivation. Inoculated into a concentrated sugar medium kept at 30° C., the fungus produces considerable amounts of citric acid. The addition of $ZnSO_4$ to such a solution increases the yield of this acid per unit of sugar utilized (**6**). This salt has a marked effect on the type of growth. The surface is covered with a thick mycelial mat, heavily wrinkled and corrugated, dipping deeply into the liquid, thus permitting greater contact between the medium and the growth. The typical black spores are missing, having been replaced by spore bodies of an olive green to gray tinge. The addition of the $ZnSO_4$ increases the growth two- to threefold (**8**). On the other hand the growth on a solution containing no $ZnSO_4$ presents a different situation. The mycelium forms a thin pellicle, profusely covered with black spores, and the heavy folds and wrinkles are absent. Such marked differences in growth induced by the $ZnSO_4$ must be due to the differences in the chemical composition of the mycelium obtained from each treatment.

Experimentation

Sufficient mycelium was grown on solutions containing 10 per cent sugar and harvested. One liter of solution A contained: sugar, 100 gm.; $NaNO_3$, 4.0 gm.; K_2HPO_4, 1.0 gm.; $MgSO_4.7H_2O$, 0.5 gm.; $FeCl_3$, 0.02 gm. Solution B had 0.1 gm. $ZnSO_4$ in addition.

The media were distributed to a depth of 4 to 5 cm. in Erlenmeyer flasks, and after sterilization were inoculated with a spore suspension of *Aspergillus niger* which was previously grown on CZAPEK'S agar (**3**). After 7 days' incubation at 28° C. the pellicles were re-

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

moved, washed in three changes of distilled water, and dried at 70°–80° C. The growths were finely ground, and portions were taken for the determinations of moisture, nitrogen by Kjeldahl method, ash, carbon (**2**), and zinc. The amounts of titratable acidity, citric acid, and sugar were determined in the solution (**6**). These results have been presented in table I.

One can readily see that the presence of the zinc salt has more than doubled the yield of mycelium per unit of available sugar.

TABLE I

RESULTS OF GROWTH OF ASPERGILLUS NIGER IN 1 LITER OF 10 PER CENT SUCROSE MEDIUM

	Solution	
	A	B
$ZnSO_4$	Absent	0.100 gm.
Sugar available	100 gm.	100 gm.
Sugar utilized	49.5 gm.	77.9 gm.
Mycelium (dried at 70° C.)	10.15 gm.	22.85 gm.
Moisture content (per cent)	8.82	9.63
Dry mycelium (100°)	9.26 gm.	20.65 gm.
Sugar utilized per gm. of mycelium	5.34 gm.	3.77 gm.
Nitrogen available	0.6588 gm.	0.6588 gm.
Nitrogen in mycelium (per cent)	3.12	2.61
Nitrogen in mycelium	0.2955 gm.	0.5392 gm.
Carbon in mycelium (per cent)	45.82	45.58
Carbon in mycelium	4.3432 gm.	9.4119 gm.
C/N ratio	14.69	17.45
$ZnSO_4$ recovered in mycelium	Trace	0.0403 gm.
Titratable acidity per liter, N/10	2191 cc.	2487 cc.
Citric acid recovered per liter	8.65 gm.	12.16 gm.

Although the amounts of sugar and nitrogen utilized have increased, yet parts of these remained in solution. About 40 per cent of the zinc was found in the organism. There has been a high storage of energy material in the mycelium, as indicated by the carbon-nitrogen ratio, which has been widened from 14.69 to 17.45.

The two types of growth were then analyzed in more detail, following the procedure for the proximate analysis of organic matter advocated by WAKSMAN (**10**). The equivalent of 10 gm. of oven-dried material was carefully wrapped in filter paper and extracted with ether in Soxhlets for 16 hours in order to obtain the ether-soluble portion. The residue was transferred from the filter papers

into beakers and extracted with 95 per cent alcohol for one-half hour on the steam bath. This was filtered through a hardened filter paper which had previously been dried and weighed. An aliquot of the alcoholic extract was evaporated to dryness in a tared dish, dried, weighed, ignited, and weighed again in order to obtain the alcohol-soluble substances and the ash. Sugars, both total and reducing, and total nitrogen were determined in other portions of the extract.

The residue remaining after the alcohol treatment was removed from the paper and extracted with distilled water at room temperature for 24 hours. The solution was filtered through the same paper and soluble matter, ash, sugars, and nitrogen were determined in portions of the aqueous extract.

The residue left after this treatment was transferred into the original beaker and treated with hot water for one hour under flowing steam. This was filtered hot through the original paper. The residue and the paper were dried at 70° C. for several hours and then for three or four hours at 100° C. and weighed so as to obtain the weight of the residue insoluble in these various solvents. Aliquots of the solution were used to determine soluble matter, sugars, nitrogen, and ash.

The dried residue was extracted with 150 cc. of 2 per cent HCl for five hours under flowing steam. The hot extract was filtered through the original paper, washed free from acid, and the residue dried as previously to determine the weight. Reducing sugars and total nitrogen were determined on the combined filtrate and washings. The reducing sugar multiplied by 0.9 gave the hemicellulose content.

The residue, after ascertaining its weight, was carefully ground with a mortar and pestle. Two 1 gm. portions of the dried residue were treated with 10 cc. portion of 80 per cent H_2SO_4 for two hours. To each flask, 150 cc. of distilled water was then added and the flask with the contents autoclaved for one hour at 15 pounds' pressure. These extracts were filtered hot through small dried and tared filter papers, washed thoroughly with distilled water, dried (first at 70° and then at 100° C.), and weighed. Ash was determined on one of the residues by igniting it. Total nitrogen was determined on

the other residue. The weight of the residue minus the sum of weights of the ash and of the protein (nitrogen×6.25) gave the lignin in 1 gm. of material left after the dilute HCl treatment. It was necessary to multiply the weight of this lignin by the weight of the residue from the 2 per cent HCl extract to obtain the lignin content in the original 10 gm. of mycelium.

The solution and filtrate from the H_2SO_4 treatment were combined. Nitrogen and reducing sugars were determined in aliquots. The reducing sugars multiplied by 0.9 gave the cellulose content in 1 gm. of residue remaining after the 2 per cent HCl treatment. This value was multiplied by the weight of the material remaining after the dilute acid treatment to obtain the total cellulose content of the 10 gm. of mycelium.

Each analysis was run in duplicate on portions equivalent to 10 gm. of oven-dried material. The results of the detailed analyses have been compiled in table II, while in table III the data have been presented on percentage basis. This method of analysis gave a high degree of recovery and defined the differences in composition between the two types of growth.

The fractions differentiated by this method of procedure contained the organic compounds soluble in the solvents used and may be divided as follows (**10**):

1. In the *ether extract* are found the fats and oils, and also a part of the resins and waxes.

2. In the *alcohol extract* are found the waxes, resins, pigments, alkaloids, and some of the sugars.

3. In the *cold-water extract* are found the various sugars, amino acids, some proteins, various organic acids, and alcohols.

4. In the *hot-water extract* are found starches, pectins, tannins, proteins, amino acids, and other organic acids.

5. In the *dilute-acid extract* are found the carbohydrates insoluble in water but soluble in dilute acid. These are the hemicelluloses, including pentosans and hexosans, which account for the greater part of this extract, and starches. In addition there are present amino acids and proteins.

6. In the *80 per cent H_2SO_4 extract* are found those carbohydrates not soluble in dilute acids but soluble in concentrated acids and

TABLE II

PROXIMATE ANALYSIS OF GROWTH OF ASPERGILLUS NIGER (ON OVEN-DRY BASIS) GROWN UPON 10 PER CENT SUCROSE MEDIA

	GRAMS	
	$ZnSO_4$ ABSENT	$ZnSO_4$ PRESENT (0.1 GM. PER LITER)
Weight of dry sample taken	10.00	10.00
Ether extract	0.269	0.518
Nitrogen	0.003	0.006
Alcohol extract	1.135	1.290
Reducing sugars	0.154	0.154
Total sugars	0.185	0.207
Nitrogen	0.022	0.027
Ash	0.004	0.007
Cold-water extract	0.523	0.884
Reducing sugars	0.045	0.042
Total sugars	0.137	0.225
Nitrogen	0.016	0.018
Ash	0.193	0.180
Hot-water extract	0.577	0.530
Reducing sugars	0.035	0.029
Total sugars	0.077	0.060
Nitrogen	0.023	0.016
Ash	0.027	0.044
Weight of residue	6.881	6.607
2 per cent HCl extract		
Reducing sugars	2.350	3.475
Nitrogen	0.104	0.075
Weight of residue*	4.082	2.729
80 per cent H_2SO_4 extract		
Reducing sugars	0.303	0.453
Nitrogen	0.013	0.009
H_2SO_4 residue	0.629	0.524
Nitrogen	0.026	0.031
Protein	0.163	0.194
Ash	0.001	0.003
Lignin (by difference)	0.465	0.327

* Residues ground and 1.0 gm. samples taken for analysis.

which give glucose as the product of hydrolysis. These include the celluloses. Amino acids and proteins are also found in this extract.

TABLE III

PROXIMATE CHEMICAL COMPOSITION OF ASPERGILLUS NIGER GROWN UPON 10 PER CENT SUCROSE MEDIA; PERCENTAGE OF DRY MATERIAL

	PERCENTAGE	
	$ZnSO_4$ ABSENT	$ZnSO_4$ PRESENT (0.1 GM. PER LITER)
Ether-soluble fraction		
Fats, etc.	2.50	4.80
Proteins	0.19	0.38
Alcohol-soluble fraction		
Waxes, sugars, etc.	9.98	11.21
Proteins	1.37	1.69
Cold-water fraction		
Sugars	4.23	7.71
Proteins	1.00	1.13
Hot-water fraction		
Starches	4.34	4.30
Proteins	1.43	1.00
Dilute HCl fraction		
Starches, hemicelluloses, etc.	20.25	31.28
Proteins	6.50	4.69
80 per cent H_2SO_4 fraction		
Cellulose	11.15	11.12
Protein	3.32	4.69
Residual material		
Lignin	18.98	8.92
Protein	6.65	5.29
Ash in the fractions	2.25	2.34
Total accounted for	*94.14*	*97.79*
Protein accounted for in fractions	20.46	15.71
Total protein (N×6.25) by analysis	19.84	16.32
Total ash	2.76	2.88

7. The *residue* from the H_2SO_4 extract contains lignin-like products and proteins not acted upon by the various treatments. As mentioned previously, the amount of lignin-like material is ob-

tained by subtracting the weight of the protein and ash found from the weight of the residue.

8. The *ash* or inorganic fraction contains the various minerals which are available for the organism.

9. The *total protein* is calculated from the nitrogen determined by the Kjeldahl method. The nitrogen found in the various fractions should equal the total nitrogen.

Discussion

As can be noted from the tables, the addition of zinc induced marked changes. In the presence of this salt 77 per cent of the available sugar was utilized, while in its absence only 50 per cent disappeared from the solution as a result of the activities of the fungus. The metabolic processes were so modified that much less energy source (sugar) was necessary to obtain per unit of mycelium. Also the yield of citric acid was increased.

The proximate analysis of *Aspergillus niger* showed that the organism, under the influence of $ZnSO_4$, stored those organic substances within its cells that may be readily utilized. The ether-soluble substances, high in energy value, were almost doubled. The yield of this fraction was more than three times greater than that obtained in the absence of zinc per unit of sugar utilized: 63 gm. sugar gave 1 gm. of fatlike substances as compared with 198 gm. of sugar per gm. of ether-soluble fraction produced in the absence of zinc. A detailed analysis of the fats was not made, but variations may be expected, for PONTILLON (**5**) showed that the degree of acidity and the nitrogen source influenced the fat fraction of fungi. It is this fraction and the alcohol-soluble fraction that play an important part in the production of ergosterol (**7**).

The alcohol fraction showed differences in the coloring matter extracted; the heavy sporulated growth gave a dark black tinge, while the zinc-treated growth imparted a distinct green tinge.

The cold-water-soluble fraction showed a marked increase due to zinc and which was probably due to the various organic acids resulting from metabolic activities. Upon cooling the hot-water extract, a gelatinous precipitate settled out which was separated, washed, and dried. This was evidently a fungus starch, dextrin-like in character,

for the iodine test gave a red color and glucosazones were obtained upon hydrolysis.

The hemicelluloses obtained by the action of dilute HCl showed that the addition of the zinc salt increased these carbohydrates by 50 per cent. In this case, this easily available source of energy constituted the largest single group of substances present in the mycelium of the fungus grown in the presence of $ZnSO_4$. The combination of this group with the nitrogenous group varied in the two fractions, as was indicated by the solubility of the proteins in dilute HCl.

The cellulose showed no differences in the two types of mycelium. On the other hand, the lignin-like substances were much less in the zinc-treated growth. This fraction is usually highest in plants that have gone through the vegetative stage and which are in the reproductive period of growth. The ratio of protein to lignin was much narrower in the growth obtained in the presence of zinc. In the insoluble material this ratio was 1.7 as compared with 2.8 in the mycelium grown on a zinc-free solution. One may gather that the lignin-like materials were formed at the expense of the hemicelluloses, for the sums of these two fractions were almost equal to each other in the two types of growth.

These analyses showed that the addition of zinc retarded the maturing of *Aspergillus niger*, holding it for longer periods in the vegetative stage and thus favoring the metabolic processes which act on and cause greater changes in the substrate.

Summary

1. *Aspergillus niger* was grown on a 10 per cent sugar nutrient solution in both the absence and presence of 0.01 per cent $ZnSO_4$.

2. The addition of this salt repressed spore formation and favored the vegetative phase of growth. Such growth showed greater utilization of the available sugar, resulting in an increased yield of the dry mycelium and a greater production of citric acid.

3. The proximate analyses showed that zinc played an important part in the nutrition of the organism, resulting in marked differences in the composition of the mycelium.

4. The addition of $ZnSO_4$ to the solution increased those fractions of the mycelium readily available to the organism, namely, the

ether fraction, the cold-water-soluble fraction, and the hemicelluloses.

5. The other fractions, with the exception of the lignin-like material, remained constant.

6. The lignin-like substances were decreased by more than 56 per cent in the zinc-treated fungus. These substances appear to be formed at the expense of the hemicelluloses.

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AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, N.J.

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PHASES OF THE ANATOMY OF ASPARAGUS OFFICINALIS[1]

CHARLES H. BLASBERG

(WITH TEN FIGURES)

Introduction

Most of the early literature on asparagus is concerned with the application of fertilizer under field conditions, with the breeding of desirable strains, and with the improvement of cultural practices without regard to the details of structure and growth of the plant. Recently NIGHTINGALE and SCHERMERHORN (**6**) made a study of the nitrogen nutrition of the asparagus plant, and there are being conducted, during the progress of this work, further experiments on the mineral requirements, the record of which will appear later.

The present work was undertaken for the purpose of obtaining a more complete knowledge of the anatomy of the plant as a foundation for a better understanding of the mineral requirements, nutrition, and of experimental selection and breeding.

Materials and methods

Seeds of *Asparagus officinalis*, of the variety Carter's Special, were planted at weekly intervals in tubs in the greenhouse. Planting was begun on March 19, 1931 and continued until August 20, 1931, about 75 seeds being planted each week. These plants were used during the anatomical study. Also 1-year-old asparagus plants were grown in pots in sand culture. These plants were used for anatomical studies as well as for microchemical and macrochemical analysis. Further to supplement the material, 2-year-old and 12-year-old plants were dug from the field when needed.

For anatomical and microchemical studies, most sections were cut free-hand. When prepared material was required, older parts of the plant (after fixation) had to be treated with hydrofluoric acid in order to remove the calcium oxalate crystals and to soften the

[1] Journal Series paper of the N.J. Agricultural Experiment Station, Department of Vegetable Gardening.

tissue sufficiently to permit sectioning. The butyl alcohol method (10) was employed throughout for dehydration.

Anatomical study

Growth habit

Asparagus officinalis is a dioecious perennial which produces large edible spears. The mature aerial stems are much branched, and reach a height of from 1 to 2 meters. Subtending each branch is a parchment-like scale leaf; the characteristic phyllotaxy is a $2 \div 5$ arrangement. The small needle-like branches (phyllodes or cladophylls), which are the chief photosynthetic organs of the plant, occur in whorls at the nodes of the stem and branches (fig. 10).

The number of stems or spears produced by any given plant varies considerably. Schermerhorn (7) reported that many of the plants in his experimental plots had to be cut nearly every day during the cutting season. These plants produced 100 or more spears each. Other plants, which were receiving the same treatment, produced only four or five spears each during the cutting season. He concluded that the ability to produce spears is apparently inherent and is not entirely due to cultural practices or to the sex of the plant.

The root system consists largely of fleshy, unbranched storage roots which vary in thickness from 2 to 6 mm. according to age, nutrient supply, and genetic composition. In general, plants which produce large spears have thick roots. The life of a storage root is not definitely known. Jones and Robbins (4) report a root which was at least 4 years old. Presumably the length of life depends upon nutrition and genetical composition. Loisel (5) claims that the plant is constantly rejuvenated by the formation of new roots as the old ones die. As the storage roots die, the gradual decomposition of the inner cortical cells results in a condition of flaccidity. The stele and the suberized outer cortical cells remain intact for some time.

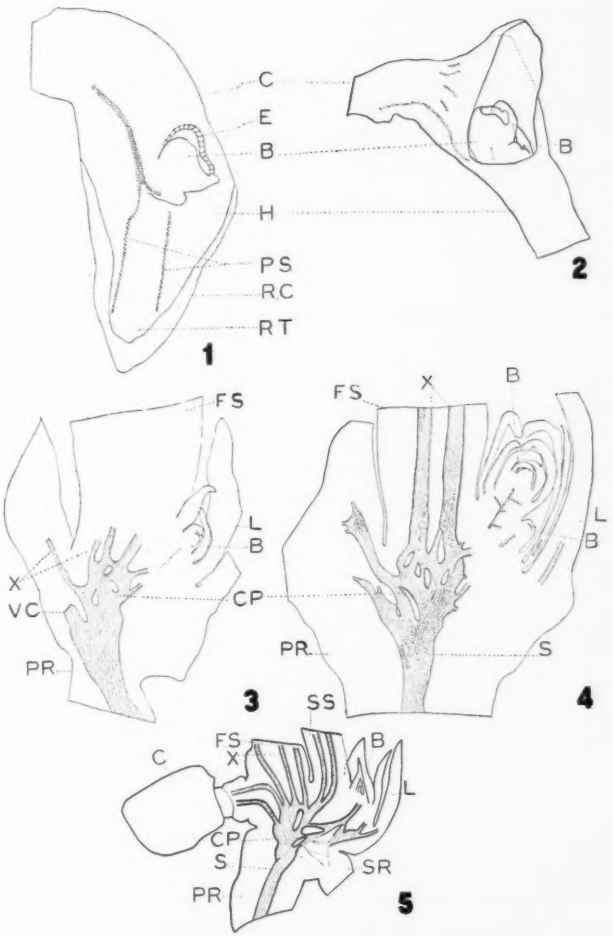
Since storage roots are produced so prolifically they soon form a thick mat in the soil, so that eventually any new roots which form must grow above the older ones. Also, as the crown continues its extension, it is mechanically inhibited by these roots from growing horizontally; consequently there is a gradual elevation of the crown until ultimately it reaches the surface of the soil.

The fibrous roots are the chief absorbing organs, and are the only part of an actively growing plant which contains nitrates (**6**), although under conditions which check vegetative growth, nitrates may be found elsewhere.

The rootstock, or crown as it is commonly called, is not a typical rhizome. It consists chiefly of the unelongated basal internodes of old stems. At the growing tip several large buds and many smaller ones can usually be distinguished. These become the aerial shoots which make their appearance above ground in a definite order, the bud nearest the last-formed spear being the next to elongate. The crowns of older plants exhibit a branching effect, for, as lateral buds develop, they extend the crown in new directions. There is a progressive development of new storage roots from the young tissue of the rootstock or crown. Most of these are ventral or lateral in origin, but occasionally one may originate from the dorsal side.

Seedling development

Seeds were germinated in the laboratory in moist petri dishes at 30° C., the optimum temperature for germination as determined by Borthwick (**2**). The hypocotyl emerged within 10 days after planting. Sections cut longitudinally through the hypocotyl showed the place of origin of the epicotyl (fig. 1). This first bud develops into the primary shoot (first spear), which usually attains a height of only 15 to 20 cm. and a diameter of about 2 mm. The second bud occurs in the axil of the basal scale leaf of the primary shoot, very slightly above the cotyledonary plate. Of the many seedlings sectioned and examined, none was observed in which the second bud originated below the cotyledonary plate as Jones and Robbins (**4**) have illustrated. This bud produces the second spear and, as shown in figure 3, it forms an angle of 180° with the cotyledon. At the base of this second spear, in some cases before it has elongated sufficiently to be called a spear, an axillary bud forms either to the right or to the left of the main growth axis; this bud produces the third spear (fig. 5). Another axillary bud may be formed at the base of the second spear but it lies in another plane (figs 3–5). Ordinarily the crowns continue the elongation during the first year along the primary axis only, in which case these lateral buds remain dormant. If the bud



FIGS. 1–5.—Fig. 1, seedling 5 days after planting, showing origin of epicotyl: *e*, epidermis; *b*, axillary bud; *h*, hypocotyl; *ps*, procambial strands; *rt*, root tip; *rc*, root cap; *c*, cotyledon; ×33. Fig. 2, seedling 10 days after planting; ×25. Fig. 3, seedling 12 days after planting: *cp*, cotyledonary plate; *fs*, first spear; *l*, scale leaf; *x*, vascular bundles; *pr*, primary root; *vc*, vascular attachment to cotyledon; ×25. Fig. 4, seedling 24 days after planting; ×25. Fig. 5, seedling 33 days after planting: *sr*, secondary root; *s*, stele; *ss*, second spear; ×10.

which forms the third spear originates to the right of the main growth axis, the bud which forms the fourth spear will be formed in the axil of a leaf to the left of the main growth axis. Thus there is an alternation of position which results in two rows of spear scars on the older rootstock. The sequential development of the buds on the rootstock forms an underground organ which is composed of many stem bases and is not a true rhizome like that of the canna.

Root structure

Storage roots.—New storage roots are formed at the base of the young, actively growing buds. They grow rapidly and may become 1 to 2 m. in length, depending upon the environmental conditions. Root hairs may be present. In the older parts of the root the epidermis, if still present, has become suberized. Although *Asparagus* is a monocotyledon, considerable increase in diameter of the storage roots is effected through cell division in the cortex. The number of cortical cells in a radial line between endodermis and epidermis varies from 35 to 50 in the older portions of the root, whereas the younger region contains only about 20 to 35 cells.

The cortex comprises by far the largest part of the storage root, the outer six to eight layers of cells being thick-walled and suberized (fig. 6). The remaining layers of cells are loosely fitted, having large intercellular spaces (fig. 6). These cells are the principal storage region of the plant. When 95 per cent alcohol is added to a section, a milky white precipitate forms which microchemical tests (**3, 8**) showed to be in part asparagose. Asparagose is an inulin-like substance which according to Tanret (**9**) yields upon hydrolysis 93 per cent fructose and 7 per cent glucose. It occurs within the cells and on the slide as minute lens-shaped crystals. The remainder of this milky fluid appeared to be chiefly a saponin. According to Tunmann (**8**) the saponins are classified as glucosides, the constitution of which has not been definitely established; but they apparently yield a sapogenin together with hexosans, pentosans, or methylpentosans. When the plants are growing actively much fructose is present, but as they approach dormancy the content of fructose decreases and the sucrose content increases. Oil or fatlike globules (**3**) are present in all parts of the plant, but occur in greatest quantity

in the endosperm cells of the seed and in the cells of scale leaves surrounding young buds. Starch is not stored in quantity, but occasional starch grains may be found in the endodermis and innermost cortical cells. Bundles of needle-shaped crystals of calcium oxalate occur in parenchymatous cells in all organs of the plant, even in the fibrous roots. The endodermis (fig. 6) has deposits of suberin and later of lignin on its radial walls, whereas the thickening of the tangential walls consists chiefly of lignin.

In a young root the cells of the pericycle are small, angular, thin-walled, and closely packed. It is in this region that the secondary or fibrous roots originate. As the root reaches maturity, the walls of the pericycle cells increase in thickness.

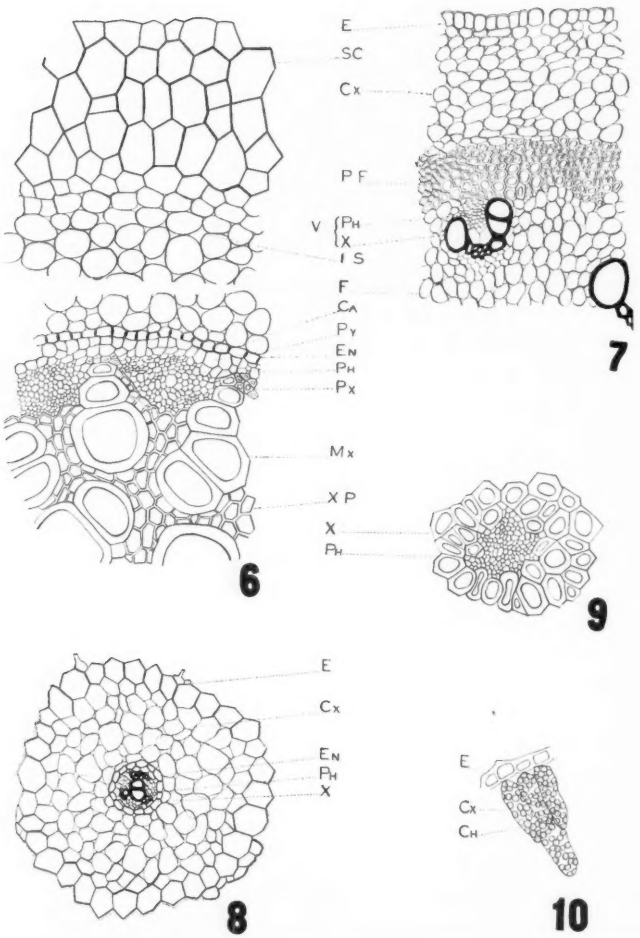
The xylem, which is all primary since there is no cambium, occupies the greatest portion of the stele. The protoxylem (fig. 6) is made up of small thick-walled cells with spiral thickenings. The number of protoxylem strands varies from 7 to 18. The metaxylem (fig. 6) has scalariform or reticulate thickenings. Each strand of xylem has one or more tracheae. The cells intervening between the xylem strands are thick-walled and lignified, so that in an old root there is a continuous cylinder of lignified tissue. Each phloem strand is composed of a few large sieve tubes and a relatively large number of small angular companion cells.

Fibrous roots.—In contrast to the storage roots, the fibrous roots are very small in diameter, usually 1 to 2 mm. The cortex is only three or five cell layers in thickness (fig. 8), and of these the outermost layer is usually suberized.

Stem structure

The 1-year-old rootstock is usually unbranched, but dormant lateral buds are present which have the potential ability to extend the crown along a new growth axis. As the dormant bud develops to form an aerial shoot, at one of its basal nodes an axillary bud is differentiated. This axillary bud will form the next spear to either the right or the left of the axis of growth. Subsequent development is similar to the development along the primary axis.

Buds are differentiated very rapidly as the plant becomes older, so that a bud just before elongation has at its basal node another



FIGS. 6–10.—Fig. 6, fleshy storage root: *sc*, suberized cortex; *is*, intercellular spaces; *en*, endodermis; *ca*, casparian strips; *py*, pericycle region; *ph*, phloem; *px*, protoxylem; *mx*, metaxylem; *xp*, xylem parenchyma; ×138. Fig. 7, cross-section through base of spear: *e*, epidermis; *cx*, cortex; *pf*, pericycle fibers; *v*, amphivasal vascular bundle; *x*, xylem; *f*, fundamental tissue; ×138. Fig. 8, cross-sections of fibrous root: *e*, remains of epidermis; ×138. Fig. 9, amphivasal bundle from crown; ×138. Fig. 10, cells from phyllode: *ch*, chloroplast; ×200.

bud which subsequently forms the next spear. This development is carried still further, so that the second bud has an axillary bud arising from it. This succession of buds gives the appearance of a cluster at the growth terminal of the crown. The number of buds is variable. Young seedlings planted March 18, 1931 had four buds in the cluster on August 25, 1931. One-year roots planted March 19 had nine buds in a cluster on August 10. The size of the bud immediately prior to its elongation is an index to the size of the spear. Large buds produce large spears and small buds, small spears. The buds just before elongation apparently contain the spear in miniature form. Longitudinal sections cut through young buds before elongation gave evidence of 17 to 20 scale leaves. Counts were made on the most luxuriantly growing stems of plants of about the same age, to determine the total number of scale leaves. The average was about 36 scale leaves per stem. Since the prepared sections showed leaves in only two of the five possible planes, the bud must have contained approximately the full number of leaves found on a mature stem. It seems logical to conclude, therefore, that at least most of the differentiation occurs prior to elongation. Elongation first begins in the lower portion of the stem, and continues upward so that the tip is still embryonic when the base is almost mature.

The epidermis of the stem contains only a few stomata and the guard cells of these contain chloroplasts. Chloroplasts are also present in the outermost cortical layers of the stem. Immediately within the cortex lies a cylinder of heavily lignified fibers, which contribute to the support of the erect stem. The vascular bundles are numerous and are scattered through the fundamental or ground tissue. The bundles are semiamphivasal, as shown in figure 7. The crown is stemlike in structure; and of the large vascular bundles which form a dense anastomosis, some are amphivasal (fig. 9) whereas others are semiamphivasal, as in the aerial portion of the stem.

The writer wishes to express grateful appreciation to Dr. RUTH M. ADDOMS and Dr. G. T. NIGHTINGALE for valuable suggestions and criticisms freely given during the progress of this work.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, N.J.

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COMPOSITION OF THE PULP AND SEEDS OF ADANSONIA DIGITATA

ROBERT A. GREENE

Introduction

Adansonia digitata, known as the baobab tree or "monkey bread" tree, is a native of the tropics. According to BAILEY (**2**) its average height is about 60 feet, but it sometimes attains a height of 90 feet, and it is said to have the thickest trunk of any tree in the world, the diameter sometimes exceeding 30 feet. The fruit, although variable in shape, is usually oblong, woody, indehiscent, and filled at maturity with a dry pulp in which there are many seeds. This pulp, known as "monkey bread," is used by natives as a food stuff. The fruit has a gourdlike structure, measuring 9 to 12 inches in length and approximately 4 inches in diameter.

This study was undertaken to determine the composition of the pulp and of the seeds. Although various investigators have discussed the uses of the fiber for various purposes, apparently the only study of the composition of the fruit has been made by PELLY (**6**).

Experimentation

The fruit was cut and the pulp and seeds separated from the remainder by means of a sieve. The seeds and pulp were then ground separately, and all determinations were made upon these samples. The relative weights of the shell, pulp, and seeds were approximately: shell, 45 per cent; pulp, 15 per cent; seeds, 40 per cent.

Both the seeds and the pulp were analyzed by the conventional methods for forage analysis, using the methods of the Association of Official Agricultural Chemists (**1**). The results are given in table I. These results show that the pulp contains a large percentage of carbohydrates, so the various carbohydrate fractions were determined, using the method of WAKSMAN and STEVENS (**7**), and the results are shown in table II.

PELLY found that most of the pulp was soluble in water, yielding

a mucilaginous liquid and an insoluble residue of cellular tissue. The aqueous extract possessed an acid taste and reaction. The acidity was attributed to free citric acid (which he identified), traces of malic acid, and acids of the pectic type, which probably occurred as acid potassium salts.

TABLE I

FEED ANALYSIS (AIR-DRY MATERIAL)

	PERCENTAGE	
	PULP	SEEDS
Moisture	7.65	8.48
Ash	6.36	3.73
Protein	5.94	11.56
Fat	0.24	6.80
Crude fiber	14.25	23.56
Nitrogen-free extract	65.56	45.87

TABLE II

PROXIMATE ANALYSIS OF PULP (MOISTURE-FREE BASIS)

	PERCENTAGE
Ether-soluble organic matter	0.26
Alcohol-soluble organic matter	1.26
Cold water-soluble organic matter	50.96
Hot water-soluble organic matter	5.08
Hemicelluloses	8.25
Celluloses	9.44
Lignin	5.94
Crude protein	4.37
Ash	6.85
Total	92.41

Table II shows that approximately 50 per cent of the pulp is soluble in water. When water was first added to the material and mixed well, a gel was formed which was broken up by prolonged stirring. A highly colloidal solution resulted, which could be filtered only by suction. The filtrate was opalescent, resembling a suspension of starch. In order to determine the nature of this fraction, other determinations were made. The results are given in table III.

An examination of table I shows that the seeds contain 11.56 per cent of protein. A small portion was extracted with various solvents,

in order to determine the classes of proteins present. The method used was similar to that employed by JOHNSON (5) in his study of the chemistry of the tubercle bacilli. The results appear in table IV.

Discussion

Table I shows that the composition of the seed is somewhat similar to that of the seed of *Chenopodium album*, and also of a mixture

TABLE III

ANALYSIS OF COLD WATER-SOLUBLE FRACTION OF PULP (MOISTURE-FREE BASIS)

	PERCENTAGE
Reducing sugars (expressed as dextrose)	13.18
Protein (N×6.25)	2.01
Acid (expressed as citric)	2.95
Ash	4.13
	22.27
Undetermined	28.69
Total	50.96

TABLE IV

DISTRIBUTION OF NITROGEN IN SEEDS

	PERCENTAGE OF TOTAL N
Water-soluble	16.19
Globulin (soluble in 10% NaCl)	35.95
Glutelins (soluble in 0.2% NaOH)	5.95
Prolamines (soluble in 70% C_2H_5OH)	1.39
Other proteins (by difference)	40.52
Total	100.00

of mesquite beans and pods, although the latter are somewhat lower in fat content (4). The composition of the pulp is similar to that of wheat bran (except that the protein and fat content of the baobab pulp are lower), dried beet pulp, and bread (4). Analyses of bread, however, show a lower ash and crude fiber content and a slightly higher protein content.

The seeds, so far as analysis shows, should make a very good stock food when ground. PELLY (6) states that the seeds contain no alkaloids or cyanogenetic glucosides, but that the seed coats are so

hard that the seeds must be boiled in order to render them soft enough to be used as cattle food.

The quantity of oil present is too small to give the seeds any commercial importance as a source of oil. There is, however, an interesting economic relation between the seeds of this tree and several species of *Dysdercus*, which are the principal cotton pests of the Sudan. These insects puncture the green bolls of the cotton plant, penetrating the seeds in order to feed upon the oil. When cotton is not available these insects feed, among other things, upon the oil in baobab seeds and in this way are able to survive until another cotton crop is available.

Tables II and III show that about 50 per cent of the pulp is soluble in cold water. In table III, about one-half of the water-soluble constituents are accounted for. The figures for reducing sugars appear high, and it is doubtful whether fresh pulp contains as great an amount. Qualitative tests for starch were negative, so the carbohydrates must be stored in some other form. It is possible that hydrolysis by enzymes or by the acid of the pulp may account for this apparently high value. Another possibility is the partial hydrolysis of some of the pectic substances. PELLY states that the pulp contains pectic acids which yield furfural and mucic acid, indicating the possibility of pentoses and galactose. Galactose is recognized as a constituent of pectins; but the production of furfural is not a specific test for pentoses, since galacturonic acid can be converted to arabinose under the conditions of the test. Although the percentage of pectic substances was not determined, it is probable that they would make up approximately one-half of the water-soluble fraction. GORTNER (3) gives the percentage of pectin in apple pomace as 15 to 18 (dry matter basis), lemon pulp 30 to 35, orange pulp 30 to 40, and beet pulp 25 to 30. It does not seem unreasonable to expect the pulp of *Adansonia digitata* to contain about 20 per cent of pectic material.

The water-soluble fraction contained 0.3 per cent of nitrogen, which is approximately 32 per cent of the total nitrogen. Tests for albumin were negative, which is to be expected since plant albumins seldom occur. Although a part of this nitrogen is probably in inorganic forms, it is possible that a large part occurs in organic forms.

The figures for citric acid are lower than those given by PELLY, who found 4.4 per cent. This amount, he claimed, is of no commercial value because of the interference of pectic substances with the extraction.

Table IV shows that 10 per cent NaCl extracted the major protein fraction. It is probable then that a globulin is the principal protein of the seed. Tests for albumin which were made on the water extract gave negative results.

There seem to be no commercial uses for either the pulp or the seeds. PELLY suggested that the seeds might be used as a manure or as cattle food. He also stated that in some parts of East Africa the pulp is used to coagulate Ceara rubber latex, a property due to its acid content.

Summary

1. The pulp and seeds of *Adansonia digitata* have been analyzed, and the results agree with previous analyses of PELLY.

2. A feed analysis shows that the seeds have a composition similar to the seeds of *Chenopodium album* or a mixture of mesquite beans and pods.

3. About 50 per cent of the pulp is soluble in water. Reducing sugars, acids, proteins, and ash comprise about one-half of this amount; the remainder is probably chiefly pectic substances. Neither the pulp nor the seeds gave tests for starch.

4. The principal protein of the seeds is a globulin, which apparently has never been isolated before.

5. The tree has an economic significance since it furnishes food to carry several species of *Dysdercus* (cotton pests) through periods when cotton seeds are not available.

The writer is indebted to Dr. R. H. FORBES, of Tucson, Arizona, who suggested this problem, supplied the material, and offered many valuable suggestions.

AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF ARIZONA
TUCSON, ARIZ.

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BRIEFER ARTICLES

A DEMONSTRATION OF BASAL GROWTH[1]

(WITH ONE FIGURE)

The gladiolus has been found very suitable to demonstrate basal growth of leaves. As lecture material the results present a clear-cut illustration of the origin of growth. In the laboratory this plant is excel-

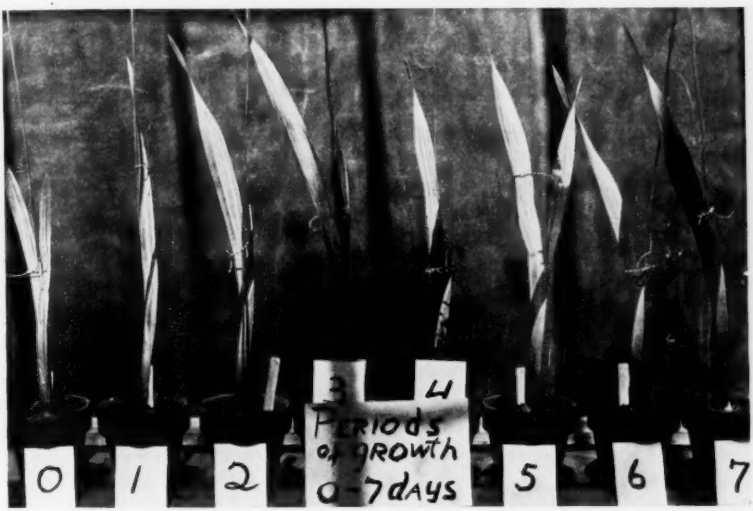

FIG. 1.—Basal leaf growth in gladiolus

lent for showing the effect of various factors on growth. An excellent contrast of old and new growth may be secured by dusting the plant with Nicotine Carrier dust. This application is a substitute for the tedious process of marking the leaf with India ink. Talcum powder may be used, either dusted or rubbed on, if the Nicotine dust is not available.

The dusting may be done when the plant has entered a vigorous growing stage. In a few hours it will be noted that new growth has broken the

[1] Approved for publication by the Head of the Department of Botany.

continuity of the dusted area, the break occurring at the point where the leaf emerges from its sheath. Figure 1 illustrates such a demonstration, showing the amount of growth in periods ranging from one to seven days. It also shows the increase in the rate of growth in the second leaf. This is particularly noticeable in the 3- and 5-day periods of the illustration.

For laboratory work the plants may be exposed to high and low temperature environments, and the growth areas measured at intervals. Table I gives the figures on the length of growth areas of plants kept for one week in cool and warm greenhouses.

TABLE I

GROWTH (IN CM.) OF GLADIOLUS LEAVES IN COOL AND WARM AIR

	PLANT NO.	FIRST LEAF (CM.)	SECOND LEAF (CM.)
Cool air	1	9.0	12.5
	2	8.5	16.4
Warm air	3	19.2	27.0
	4	21.5	26.0

This plant furnishes suitable material for the collection of data on basal growth under various conditions of time, light, and temperature. The gladiolus is not easily injured by rough handling and a sample may be used several times.

Corms harvested in the autumn will be ready for planting by February 1. If it is desired to make this demonstration in the autumn, corms should be placed in cold storage at about 35° F. by April 1. The corm germinates well if the lower half is buried in soil in a 4-inch pot.—C. J. GILGUT AND LINUS H. JONES, *Department of Botany, Massachusetts State College, Amherst, Massachusetts.*

CURRENT LITERATURE

ANNOUNCEMENT

Beginning with the current number, the BOTANICAL GAZETTE will be issued quarterly rather than eight times a year as formerly. The numbers will appear in September, December, March, and June.

This change will provide approximately the same number of pages per volume and at the same time will permit a saving in manufacturing costs. The adjustment also has enabled the publishers to reduce the amount of the annual subscription price, which is now $8.

BOOK REVIEWS

Soil conditions and plant growth

The development of soil science continues its rapid pace under the stimulus of international congresses and societies for its promotion. The most valuable English summary of the soil in its relations to plant growth during the last 20 years has been RUSSELL'S[1] excellent monograph, which has been deservedly popular throughout the long period since the first edition appeared. The sixth edition of this famous work has just come from the press.

The appearance of the new edition was delayed by RUSSELL'S study of soil technology among his Russian colleagues. The task of sorting out the most valuable new knowledge gained from these contacts, and rewriting the work to incorporate the advances of the last five years, has been a difficult one; but with the aid of the Rothamsted staff it seems to have been accomplished in admirable manner.

The book is increased about 20 per cent in size. The number of chapters and general arrangement remain the same, but chapters IV, V, and IX have been given new titles. These are now listed as: IV, The soil in nature; (1) Changes in its mineral composition; V, The soil in nature; (2) Changes in the organic matter; and IX, Methods of characterizing soils. In the preceding edition these were: Chemical and physical relationships of the soil, Carbon and nitrogen cycles of the soil, and Methods of soil examination, respectively. The main additions are made in chapter II, on soil conditions affecting plant growth; chapter III, on the composition of the soil; and chapter IV. All chapters have been increased in size except the introduction, which has been changed but slightly, and the final chapter, which has been reduced to half its former size.

[1] RUSSELL, SIR E. JOHN, Soil conditions and plant growth. 6th ed. 8vo. pp. viii+636. Longmans, Green and Co. New York and London. 1932.

The work maintains its high position as the leading exposition of the soil as a medium of plant growth. The technicalities of soil science are subordinated to an understanding of the plant in its relation to the soil. This emphasis upon the reactions of crop plants to the soil environment is what has made it so useful and so popular. It has always merited the favor it has enjoyed among students of plant behavior.—C. A. SHULL.

Phylogeny of conifers

The first part of a very important contribution to the historical development of Coniferales and Cordaitales has been published by FLORIN.[2]

The author first raises the question about morphological and anatomical characters which can be studied in the fossil material of Coniferales and Cordaitales. From there he proceeds to a detailed comparative anatomical investigation of recent conifers and discusses their applicability to fossil species. This volume is primarily devoted to the treatment of the recent forms from this point of view. The most important organ for species determination which can be examined in fossils and which, therefore, is extensively discussed in recent forms, is the structure of the epidermis of the assimilatory organs. The stomata are treated in detail for all living families of conifers. This investigation is followed by a study of the variability of the epidermis structure of fully developed assimilatory organs. He uses these characters to explain the relation between the different genera of living conifers and finally he compares the stomata of the conifers with those of the other gymnosperms. Toward the end of the volume the importance of the examination of the cuticle for the investigation of fossil plants is discussed.

FLORIN'S publication represents the first paper in a series of investigations in which comparative anatomical studies of the phylogeny of Coniferales and Cordaitales are to be carried out. The author extends his studies also upon the cuticles and stomata of other gymnosperm groups, as Ginkgoales, Cycadales, and Gnetales. This book is a step forward in our knowledge of recent, as well as fossil, gymnosperms, and the succeeding volumes should be looked for with great interest.—A. C. NOÉ.

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[2] FLORIN, RUDOLF, Untersuchungen zur Stammengeschichte der Coniferales und Cordaitales. Erster Teil. Kungl. Svenska Vetenskapsakademiens Handlingar Tredje Serien. 8vo. **10**: No. 1. pp. 588. Figs. 111; pls. 58. 1931.